



Construction of Pbs.PGK.PCR1

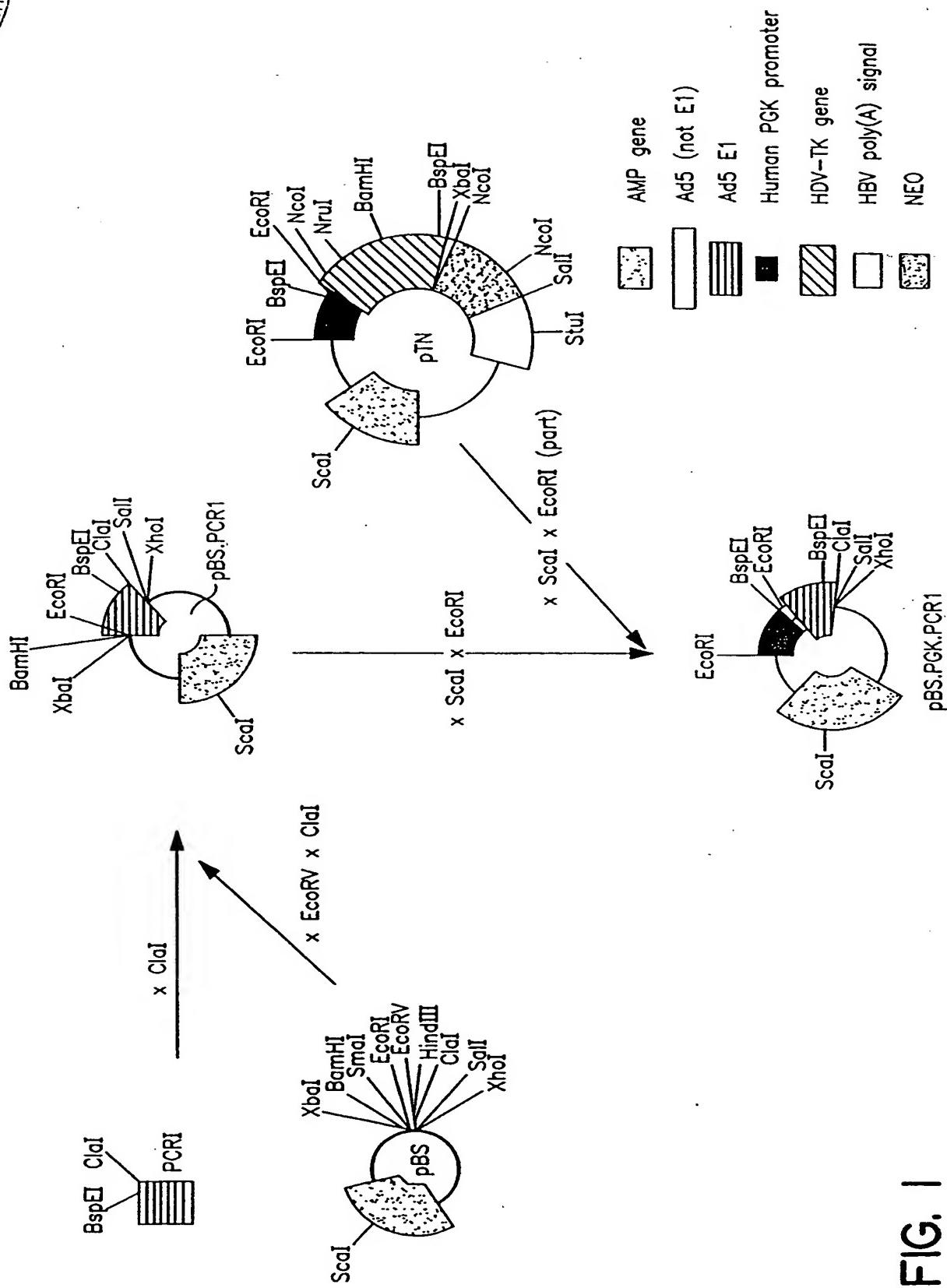


FIG. I



Construction of pIG.E1a.E1b.X

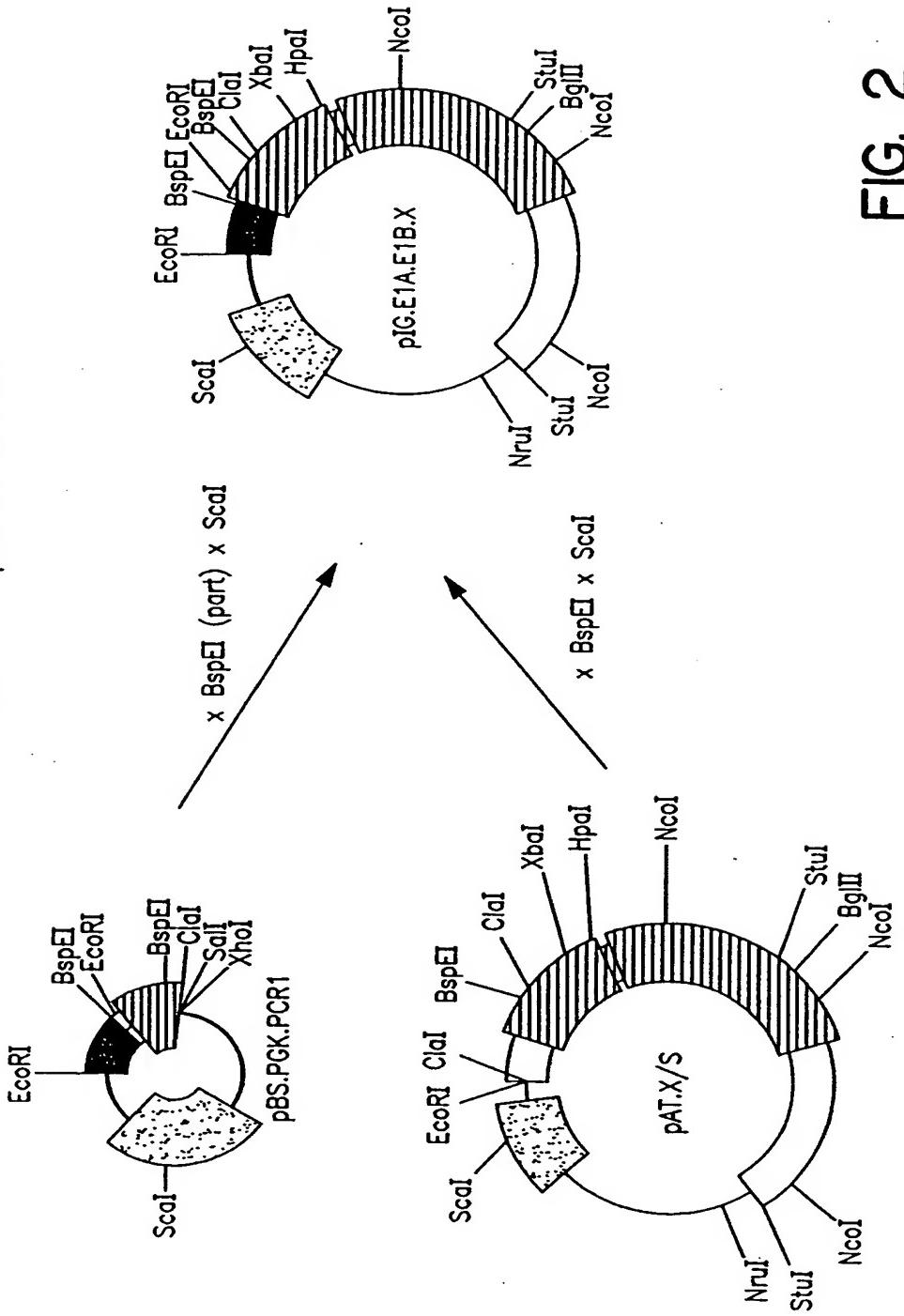


FIG. 2

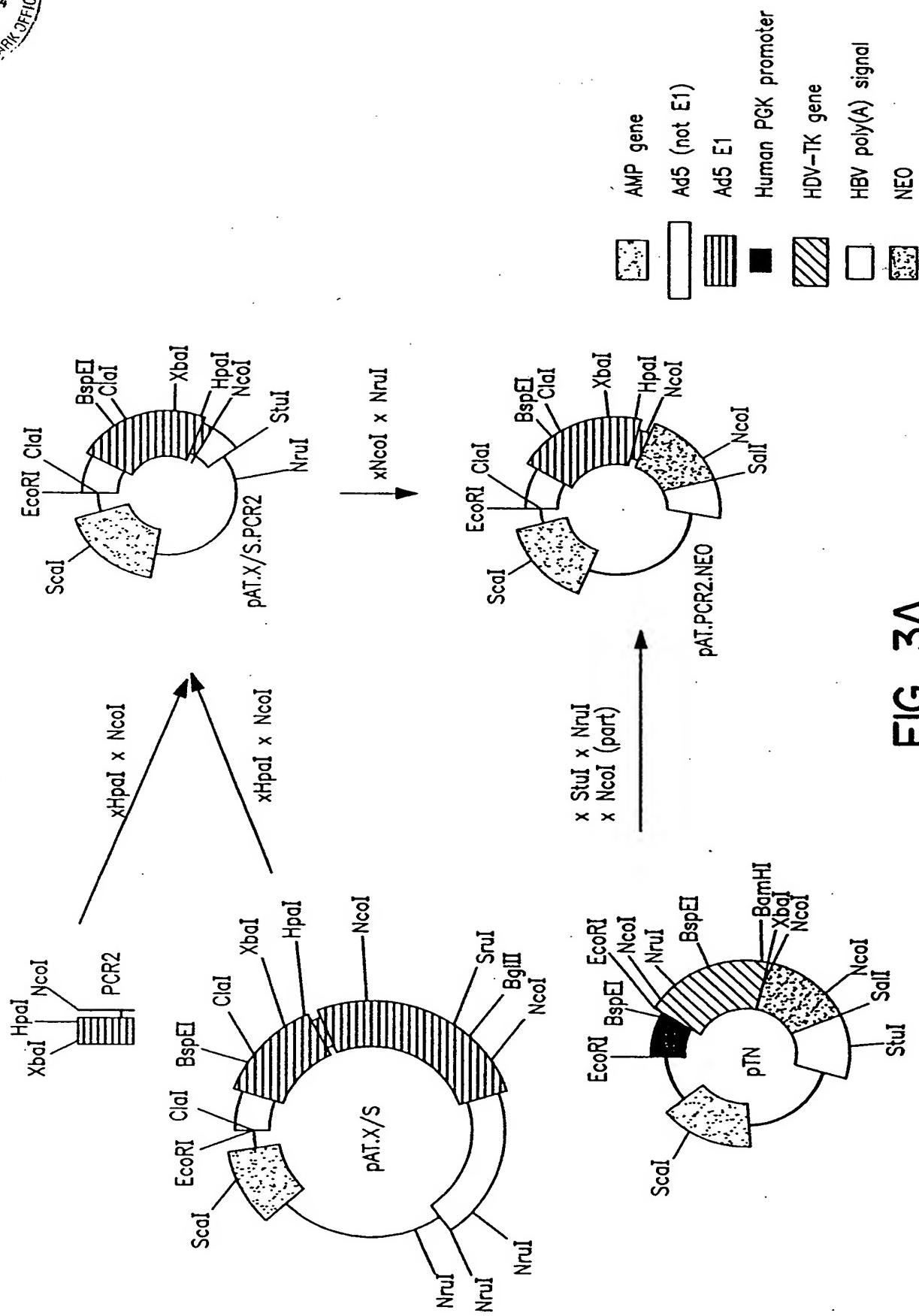


FIG. 3A



Construction of pIG.E1a.NEO

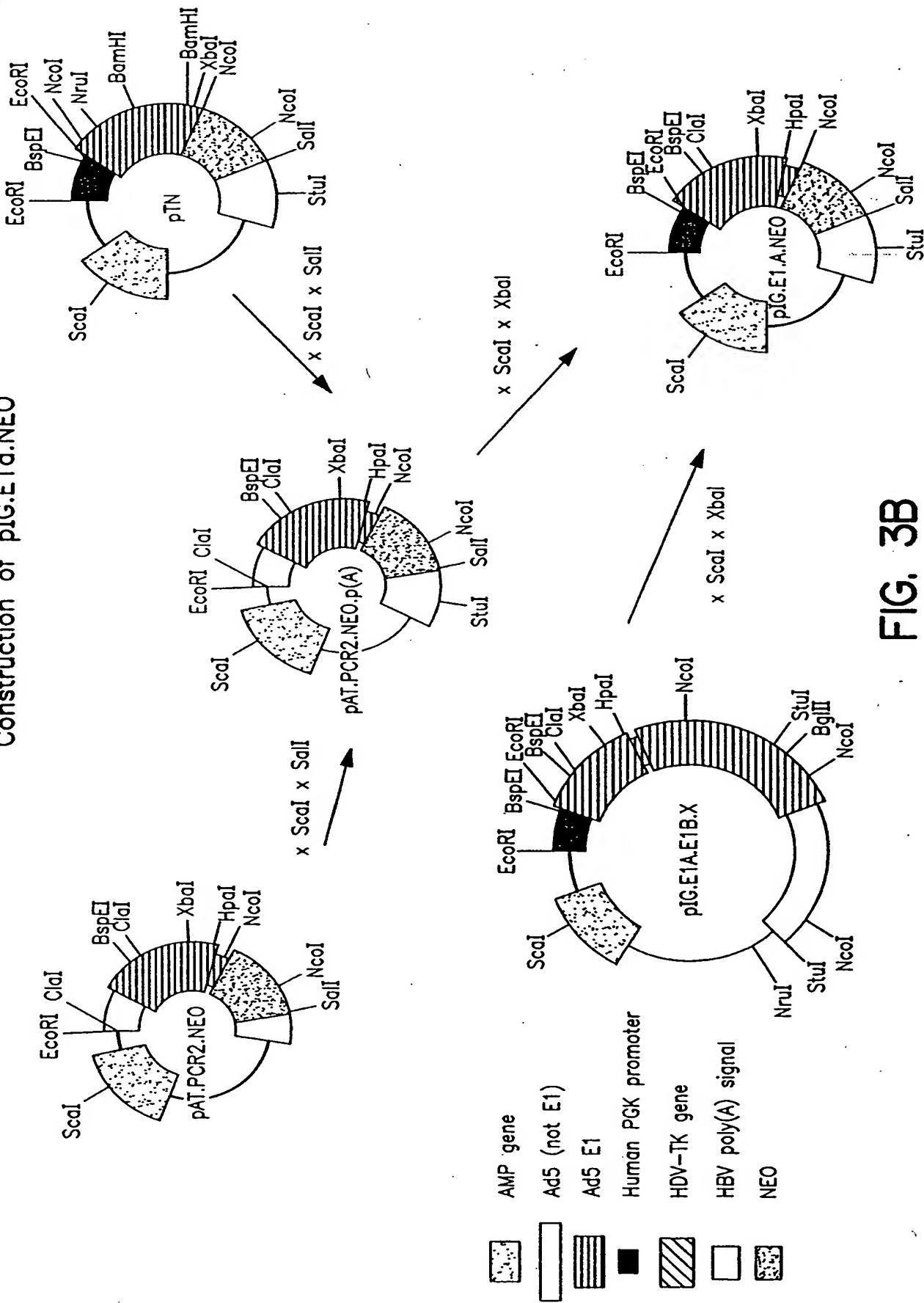


FIG. 3B

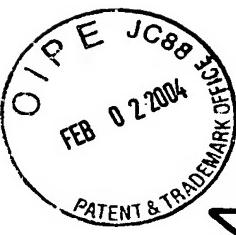
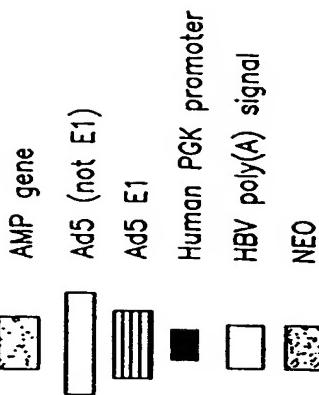
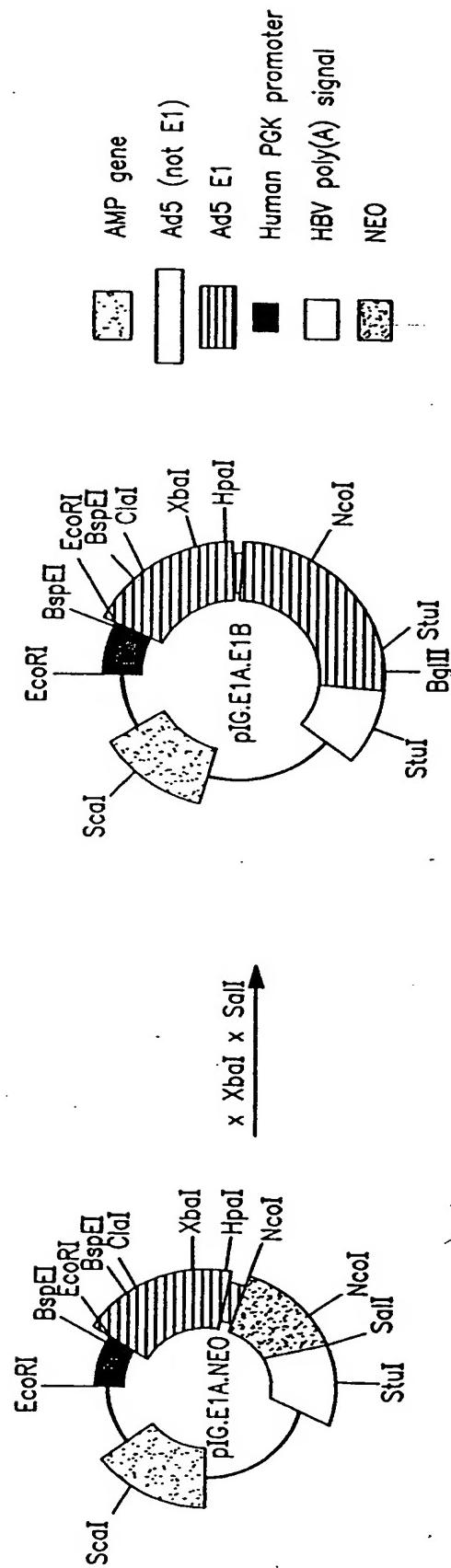
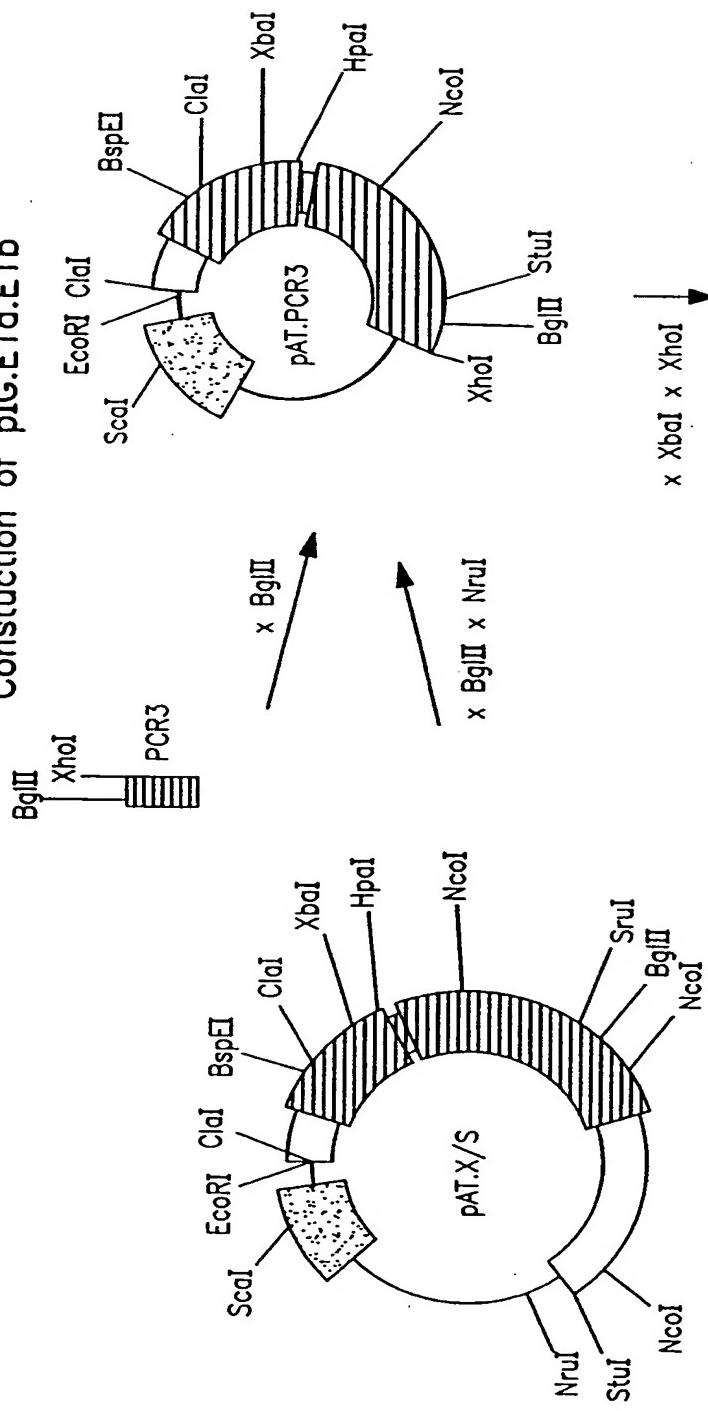


FIG. 4

Construction of pIG.E1a.E1b





Construction of pIG.NEO

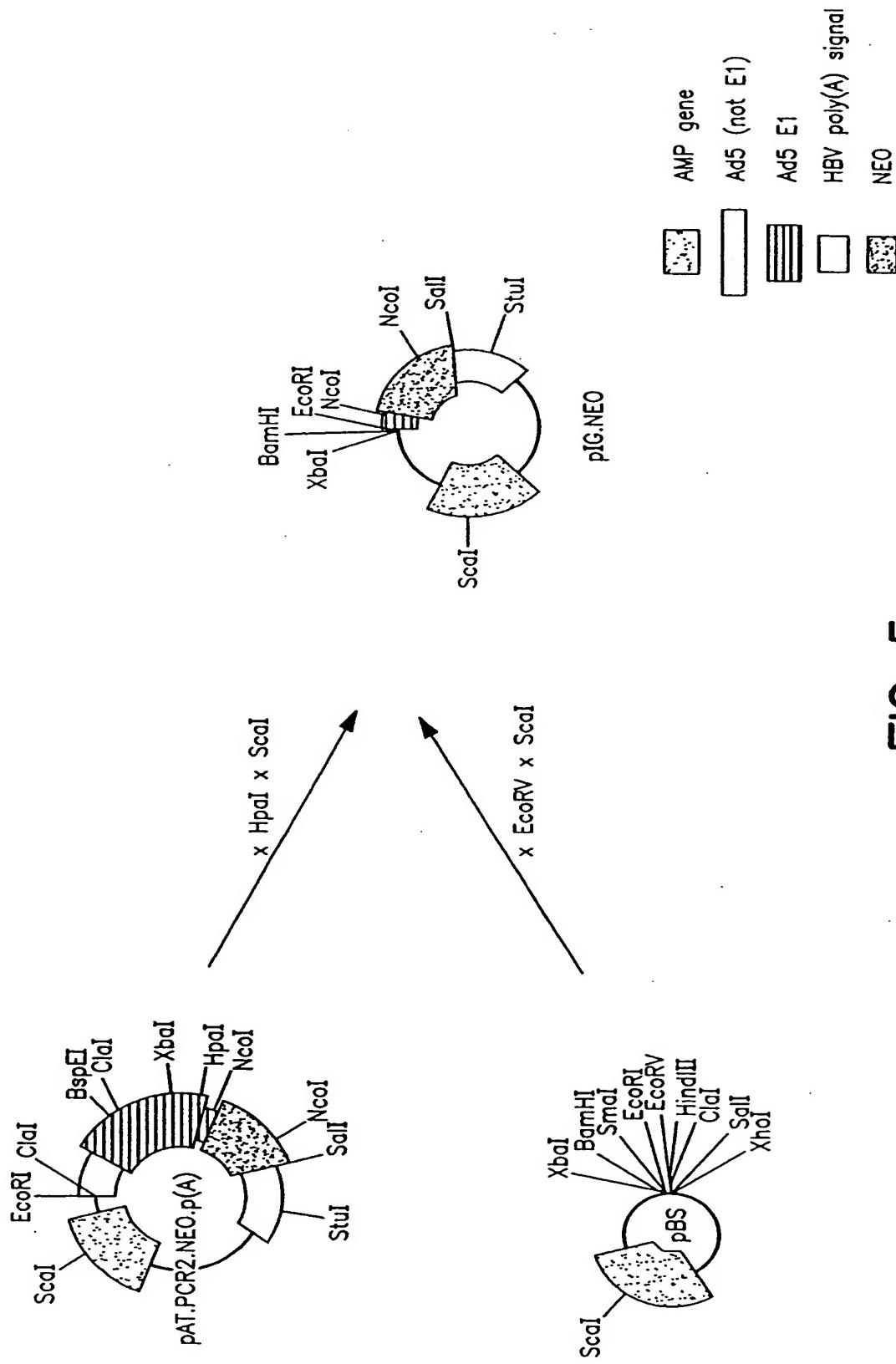


FIG. 5



Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells

transformation of primary kidney cells

$1\mu\text{g}$
 $5\mu\text{g}$



nd

nd

nd

nd

$1\mu\text{g}$
 $5\mu\text{g}$



NEO p(A)

nd

1

+ SV40.E1B (1 μg)

nd

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

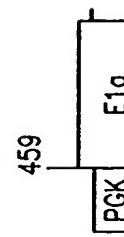
nd

nd

*average of 5 plates 21 days after transselection



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

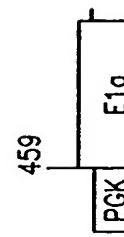
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

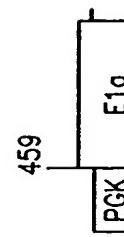
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

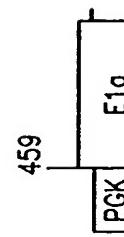
nd

nd

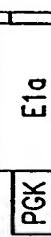
nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

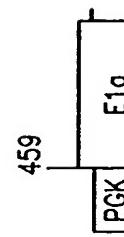
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

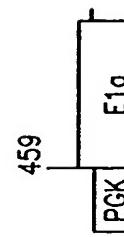
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

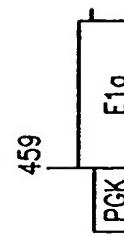
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

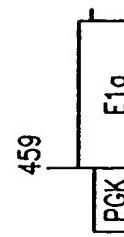
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

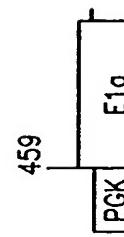
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

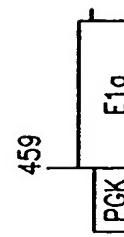
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

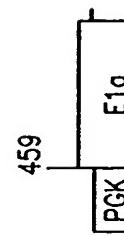
nd

nd

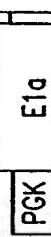
nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

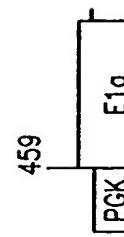
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

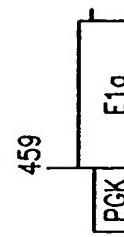
nd

nd

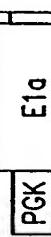
nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

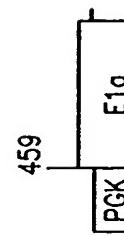
nd

nd

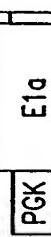
nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

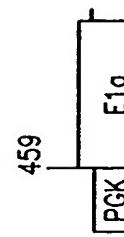
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

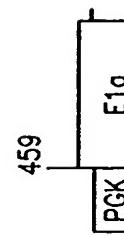
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

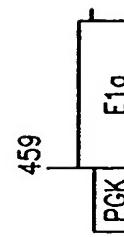
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

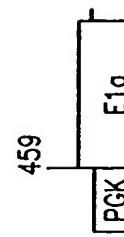
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

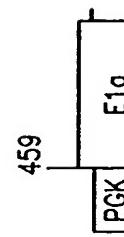
nd

nd

nd



NEO p(A)



E1b



$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

$1\mu\text{g}$
 $5\mu\text{g}$

nd

nd

nd



Western blotting analysis of A549 clones transfected
with pIG.E1A.NEO and PER clones
(HER cells transfected with pIG.E1A.E1B)

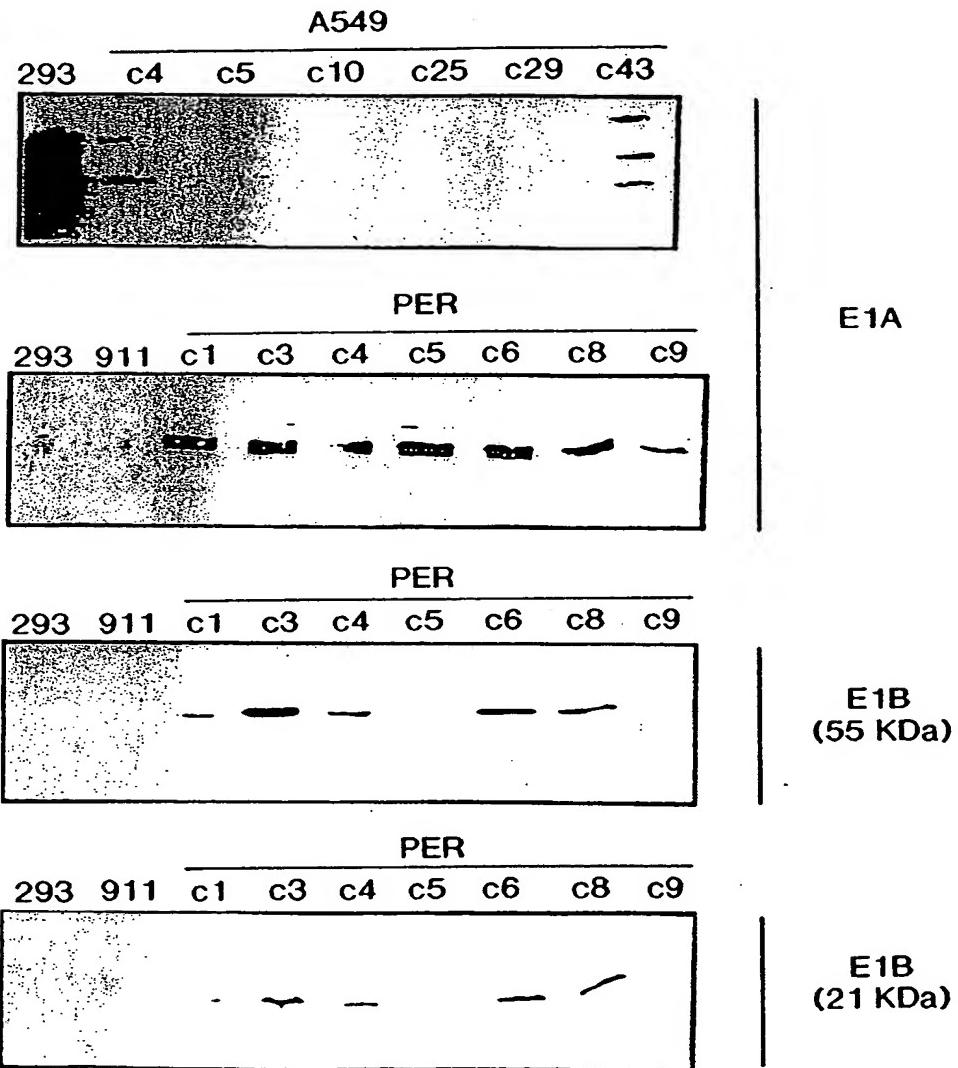


FIG. 7



Southern blot analyses of 293, 911 and PER cell lines

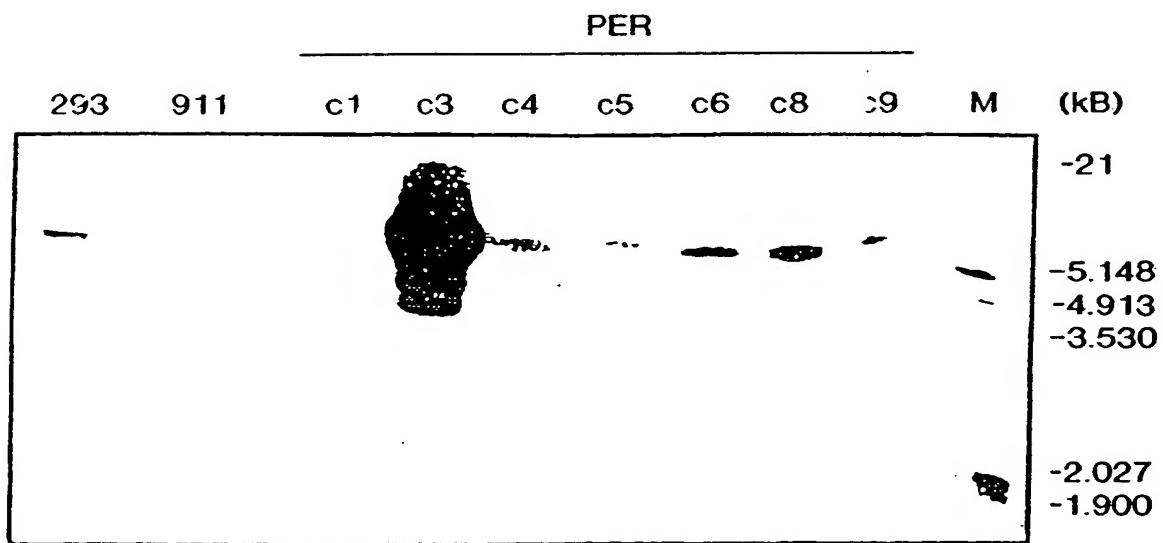


FIG. 8



Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected ($n=2$) with 5 μ g pRSV.lasZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

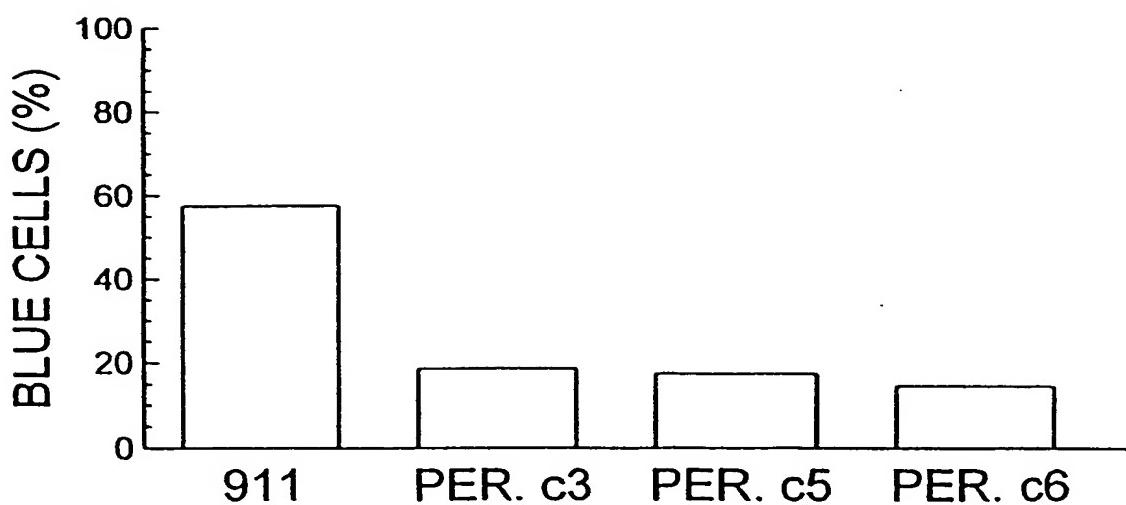


FIG. 9



Construction of pMLP1.TK

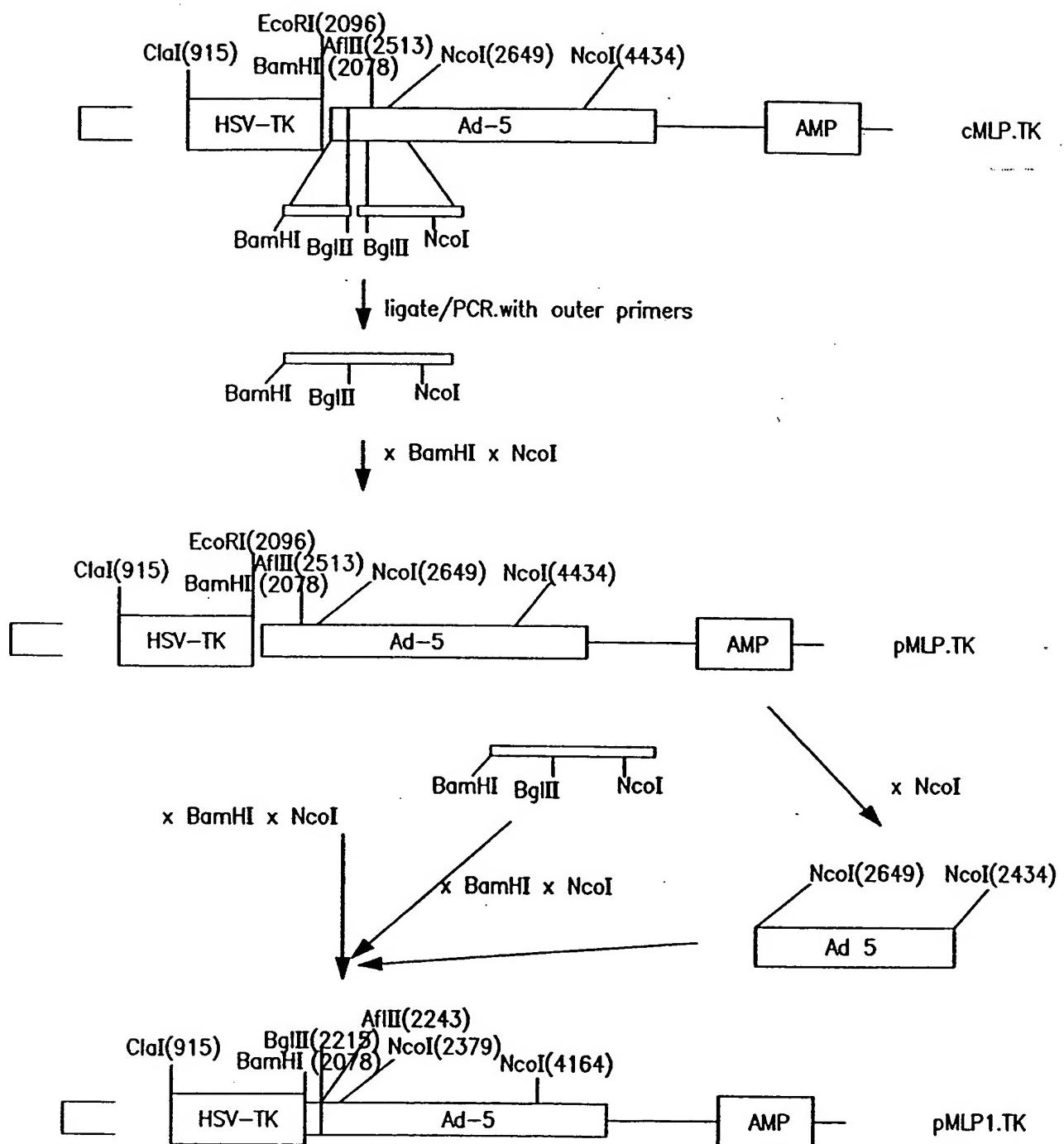


FIG. 10



New recombinant adenoviruses and packaging constructs without sequence overlap

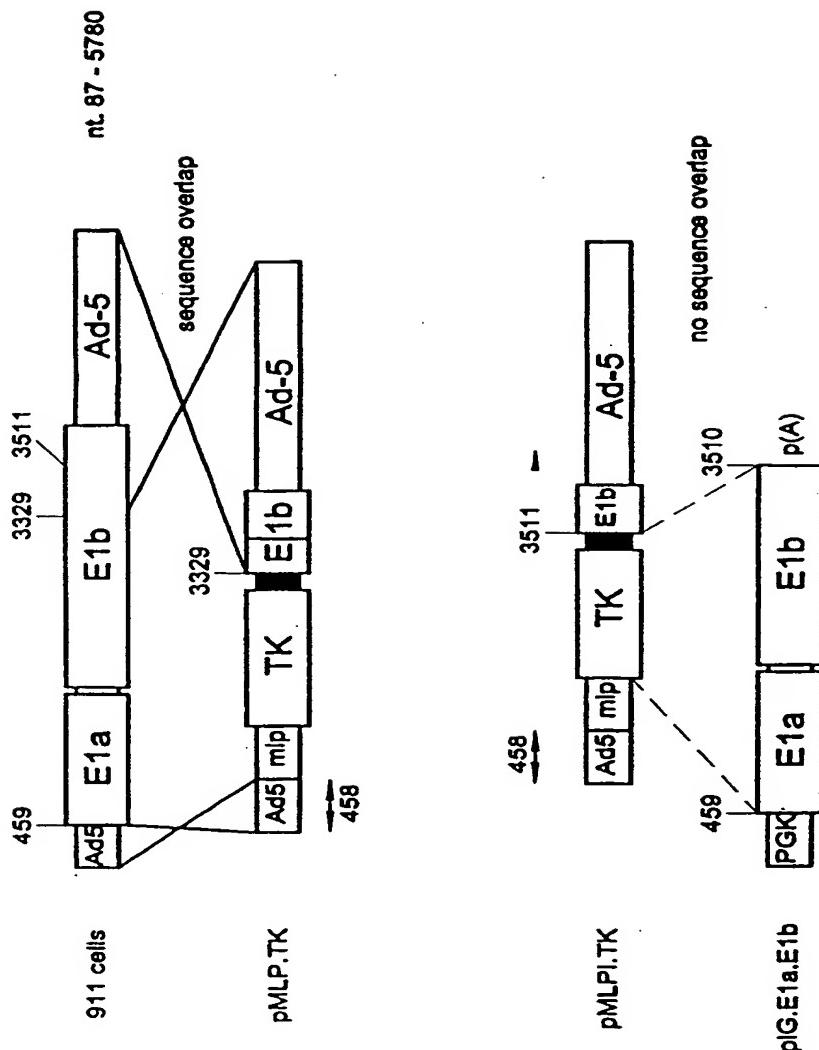
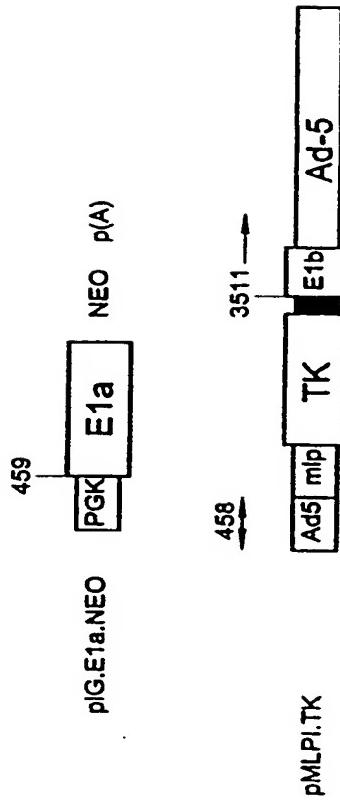


FIG. 1A

Packaging system based on primary cells



New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418
FIG. I |B



Generation of recombinant adenovirus

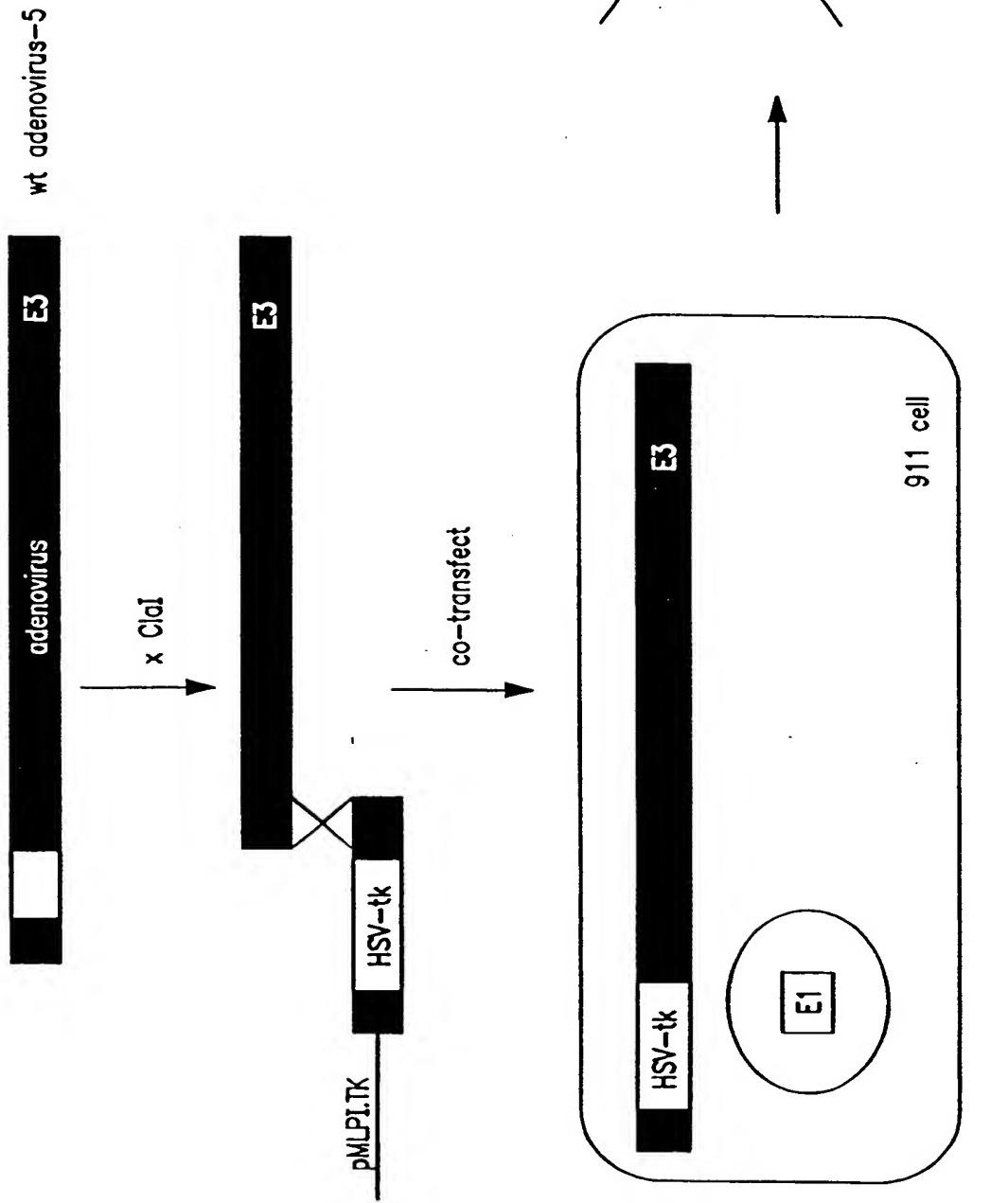


FIG. 12

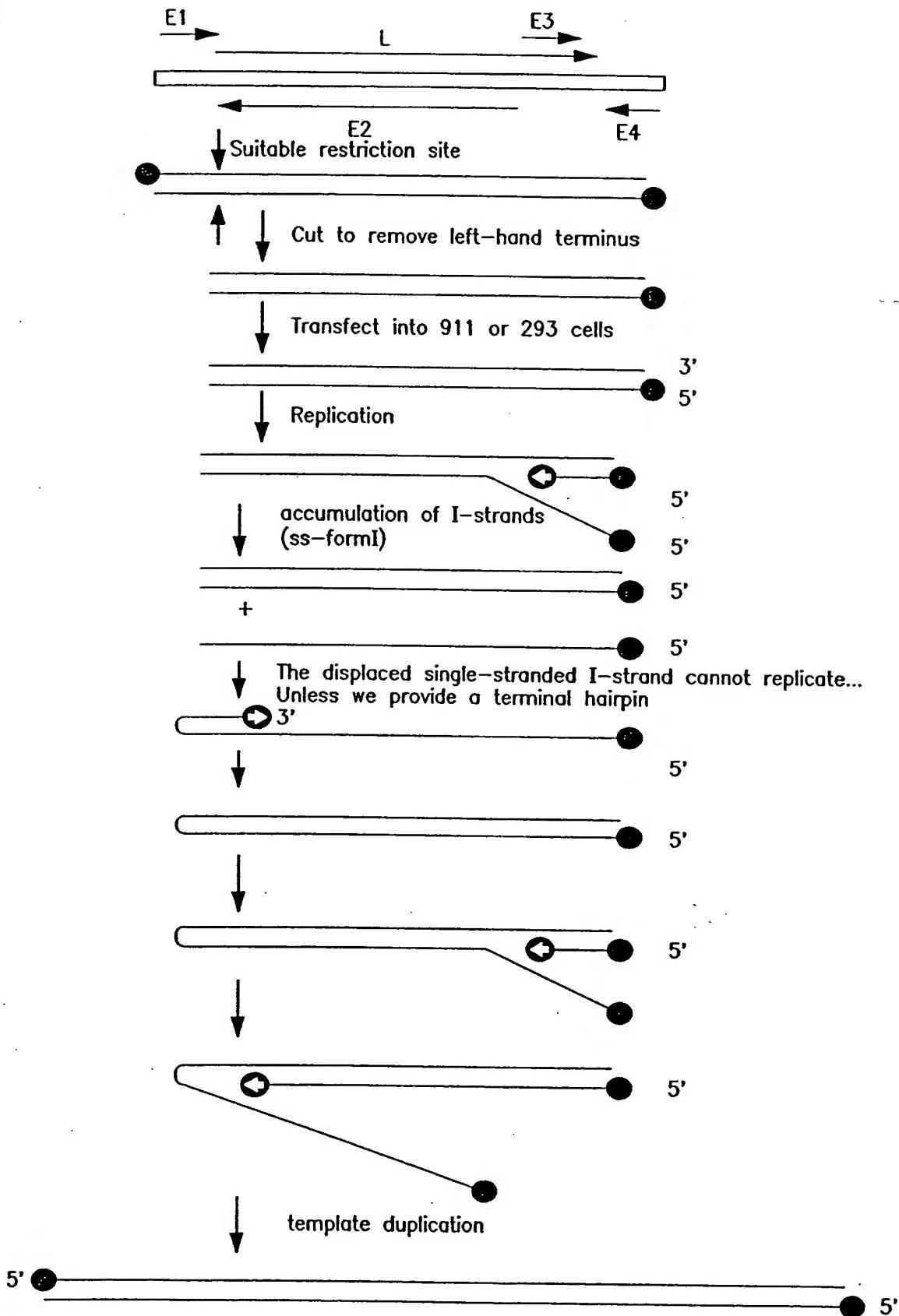


FIG. 13



Replication of Adenovirus

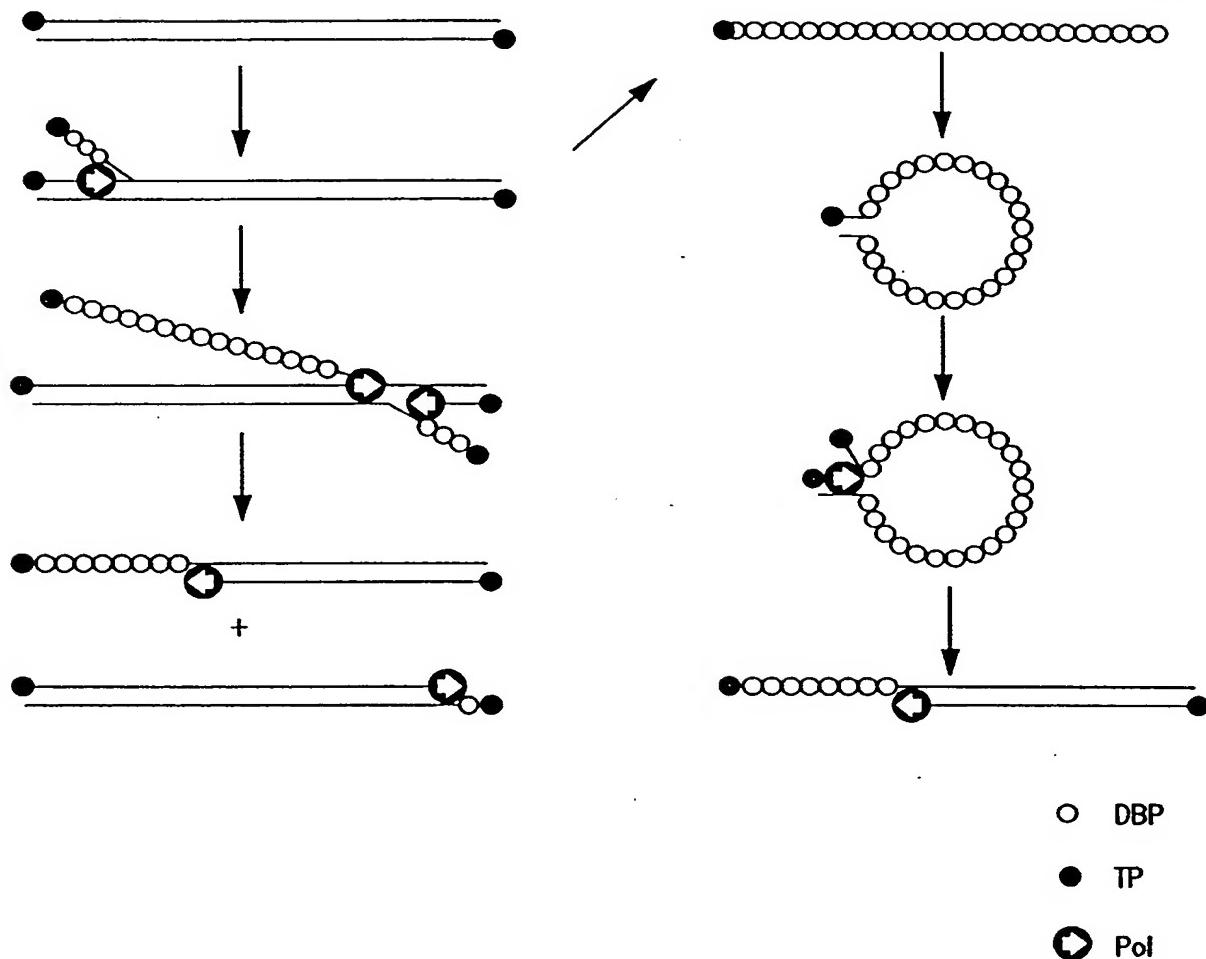


FIG. 14



The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.

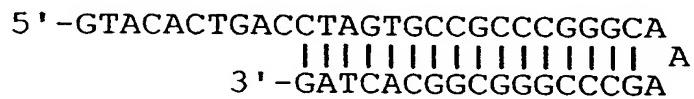


FIG. 15

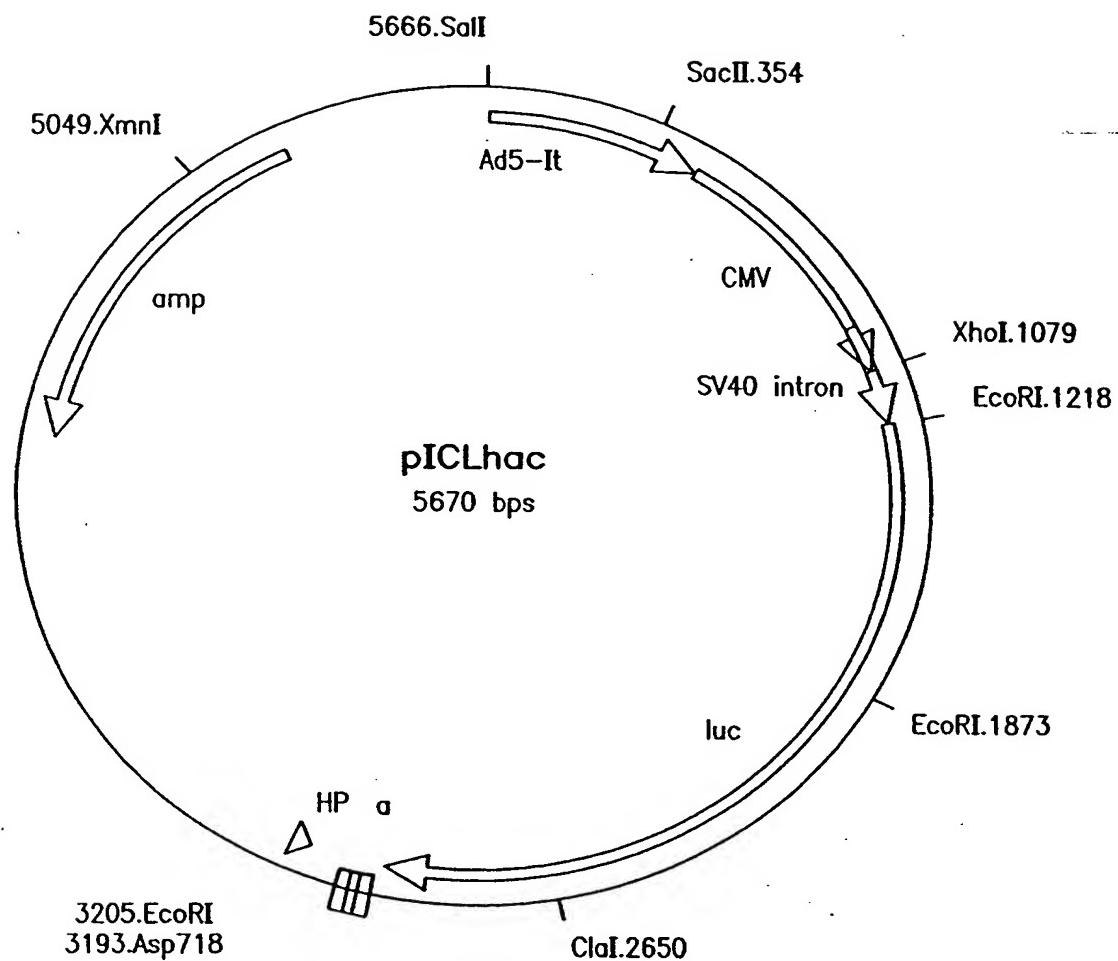


FIG. 16

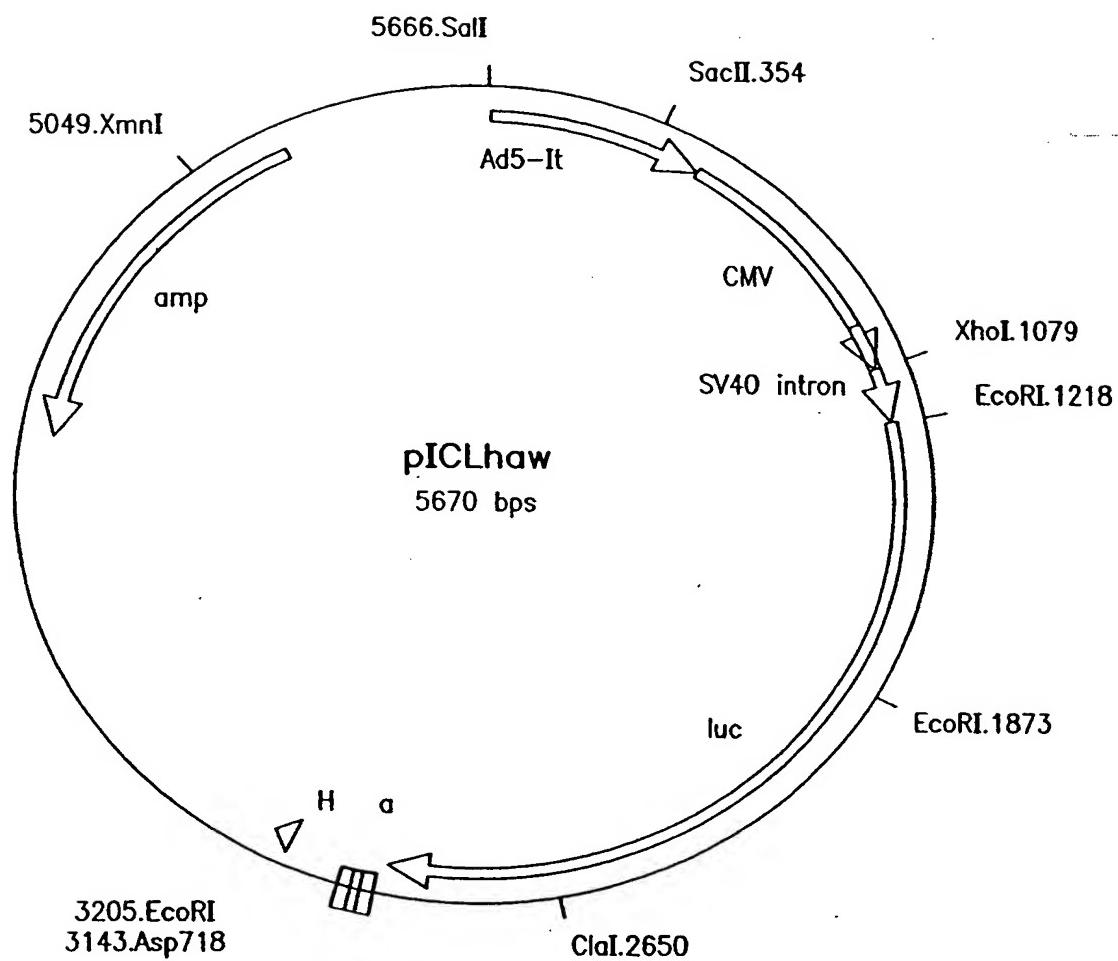


FIG. 17

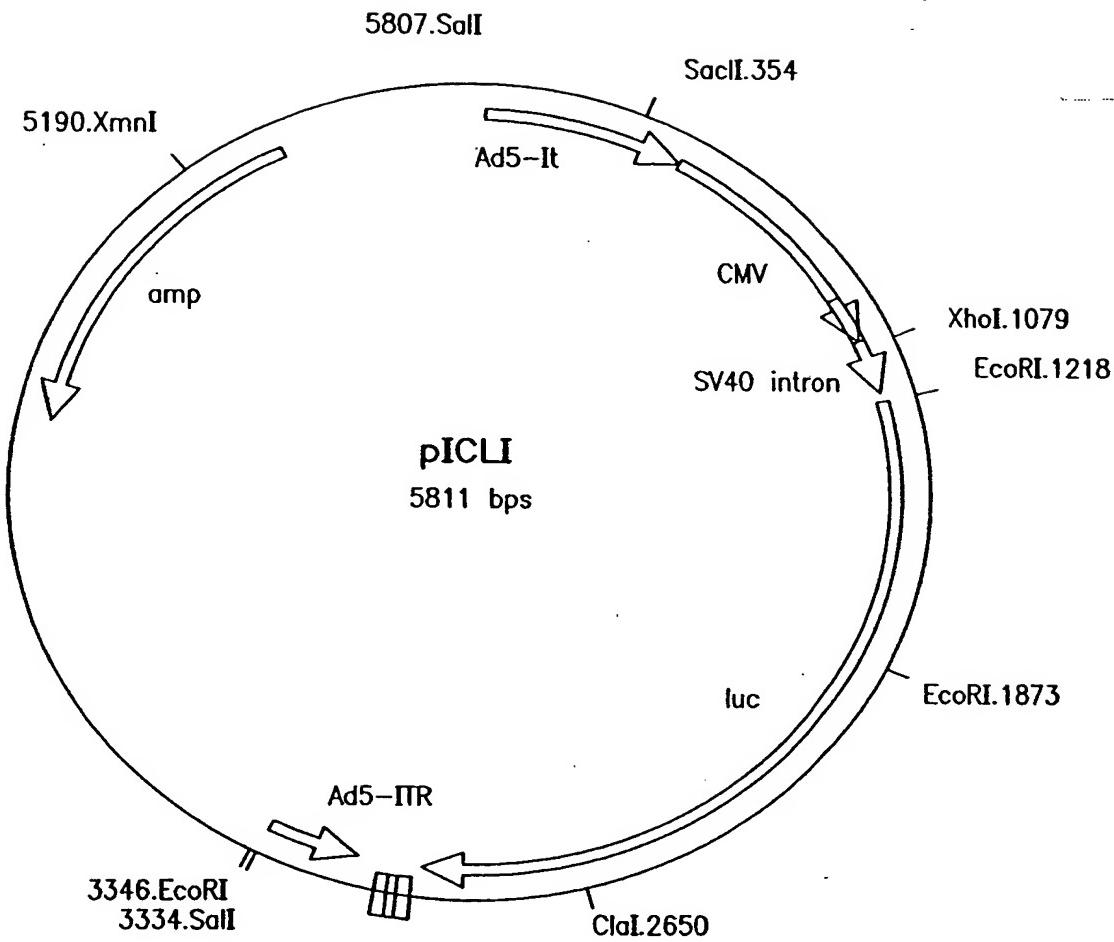
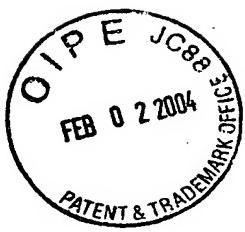


FIG. 18

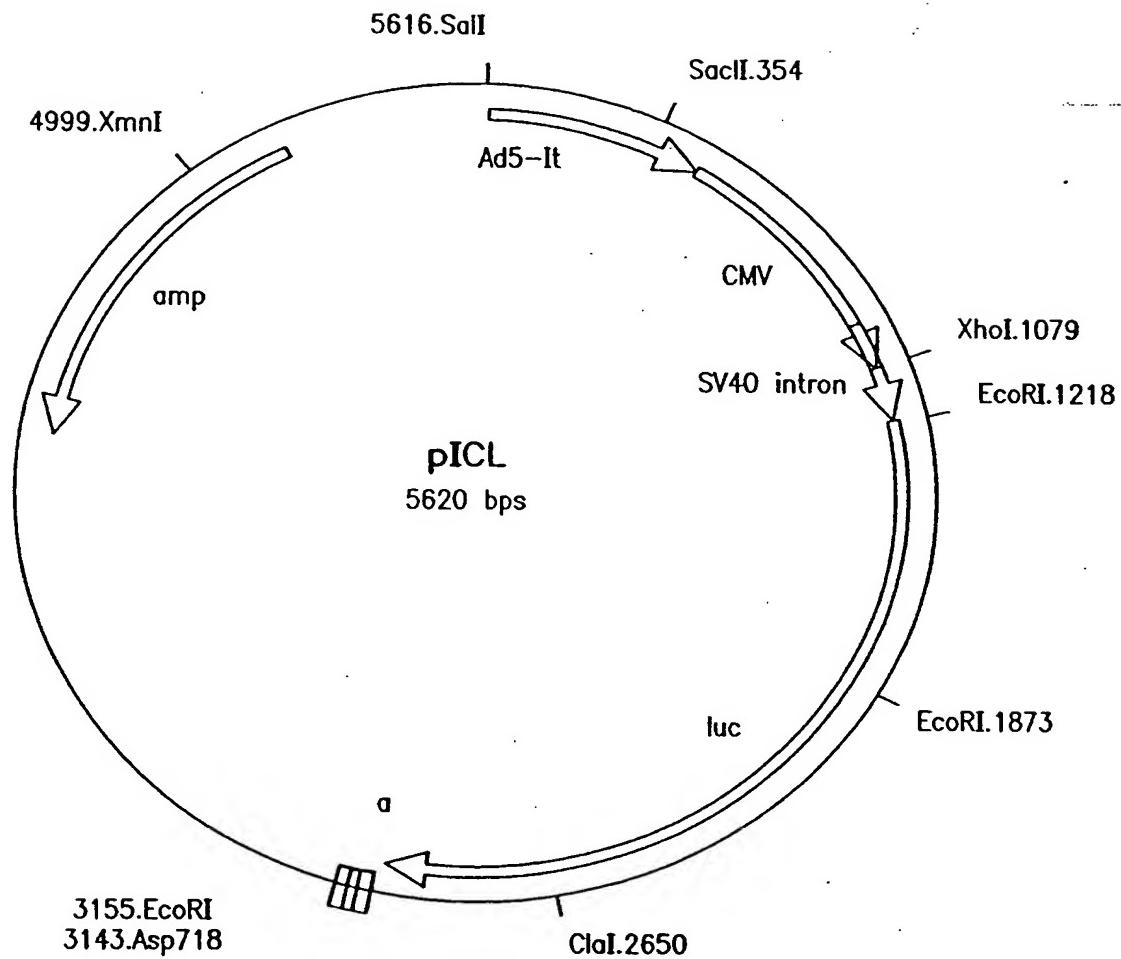


FIG. 19



Cloned adenovirous fragments

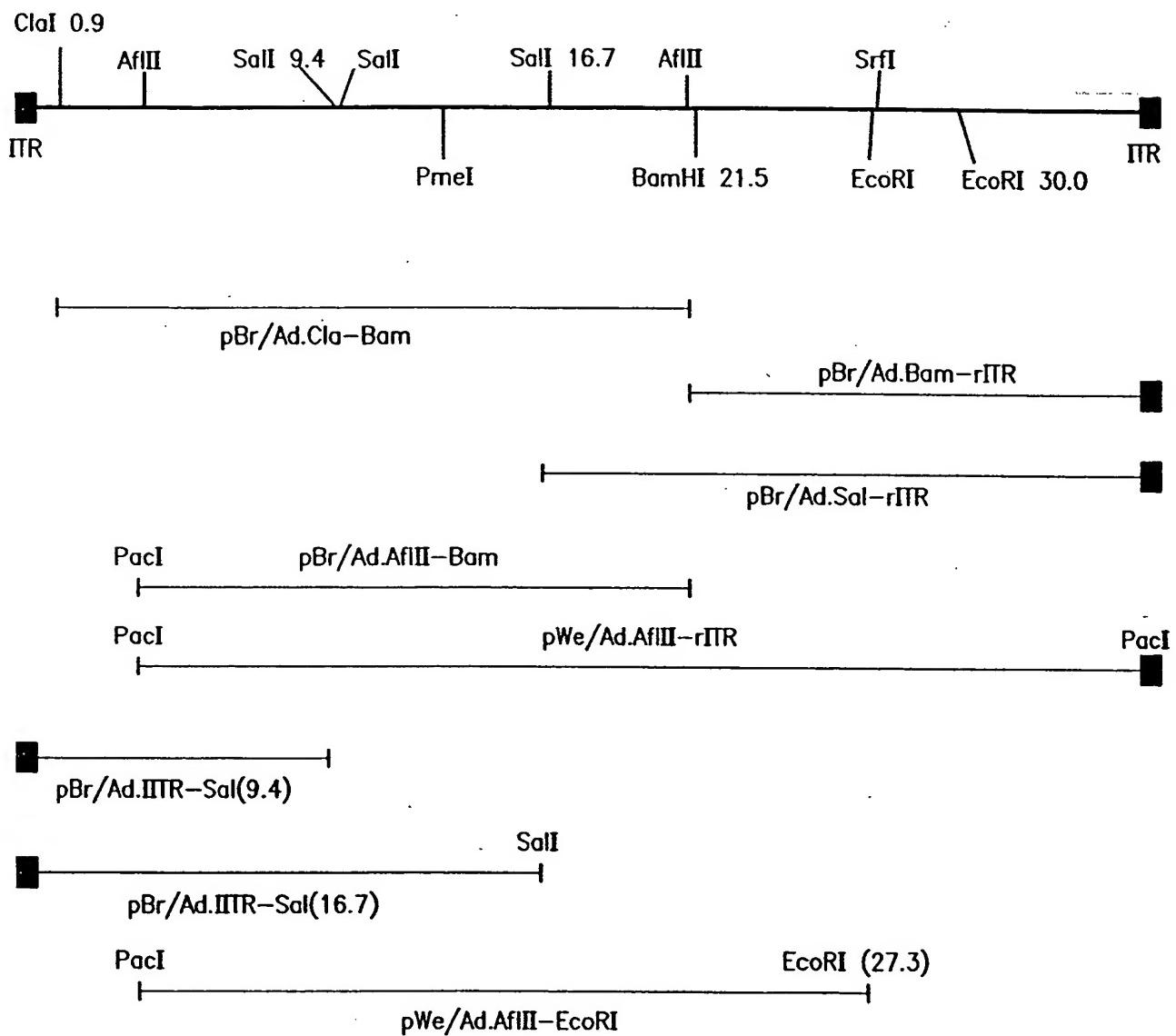
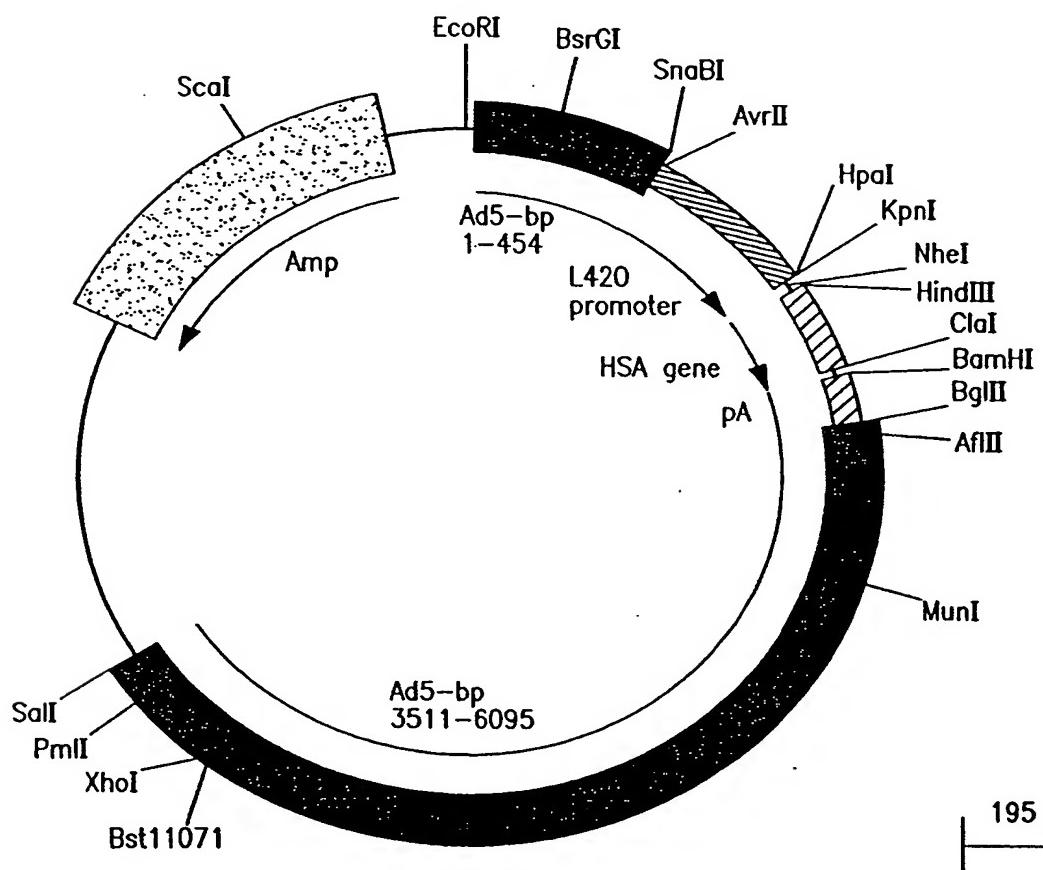


FIG. 20



Adapter plasmid pAd5/L420-HSA



195

FIG. 21



Adapter plasmid pAd5/CLIP

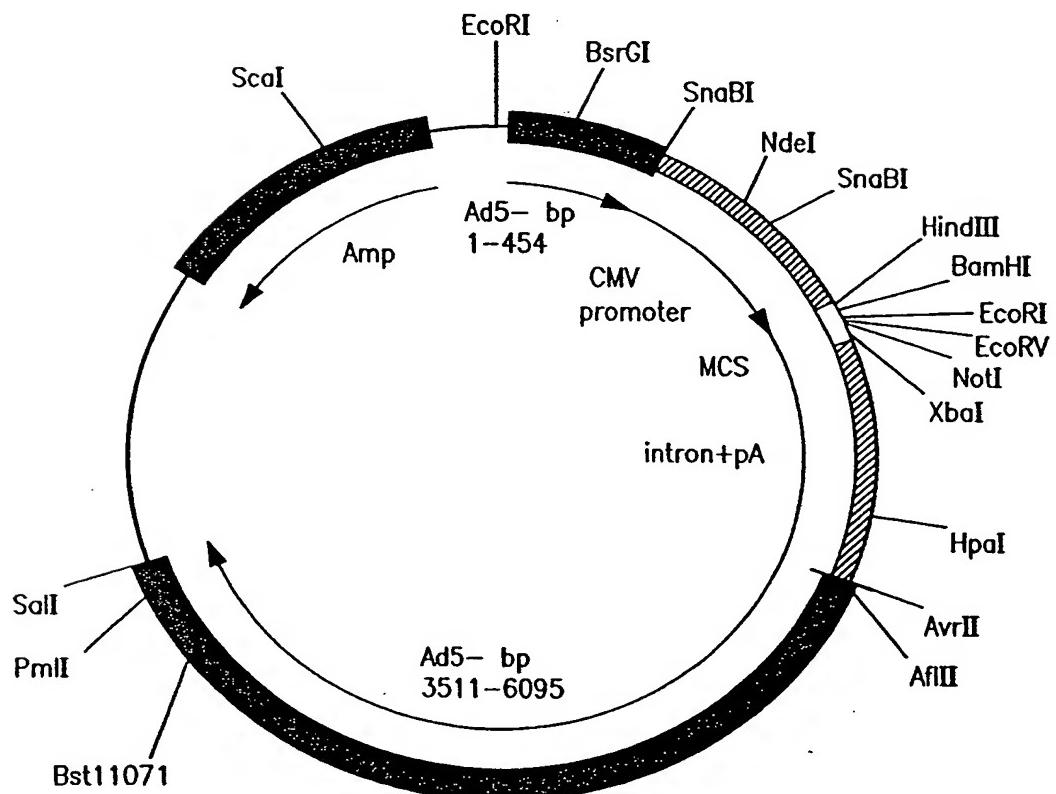
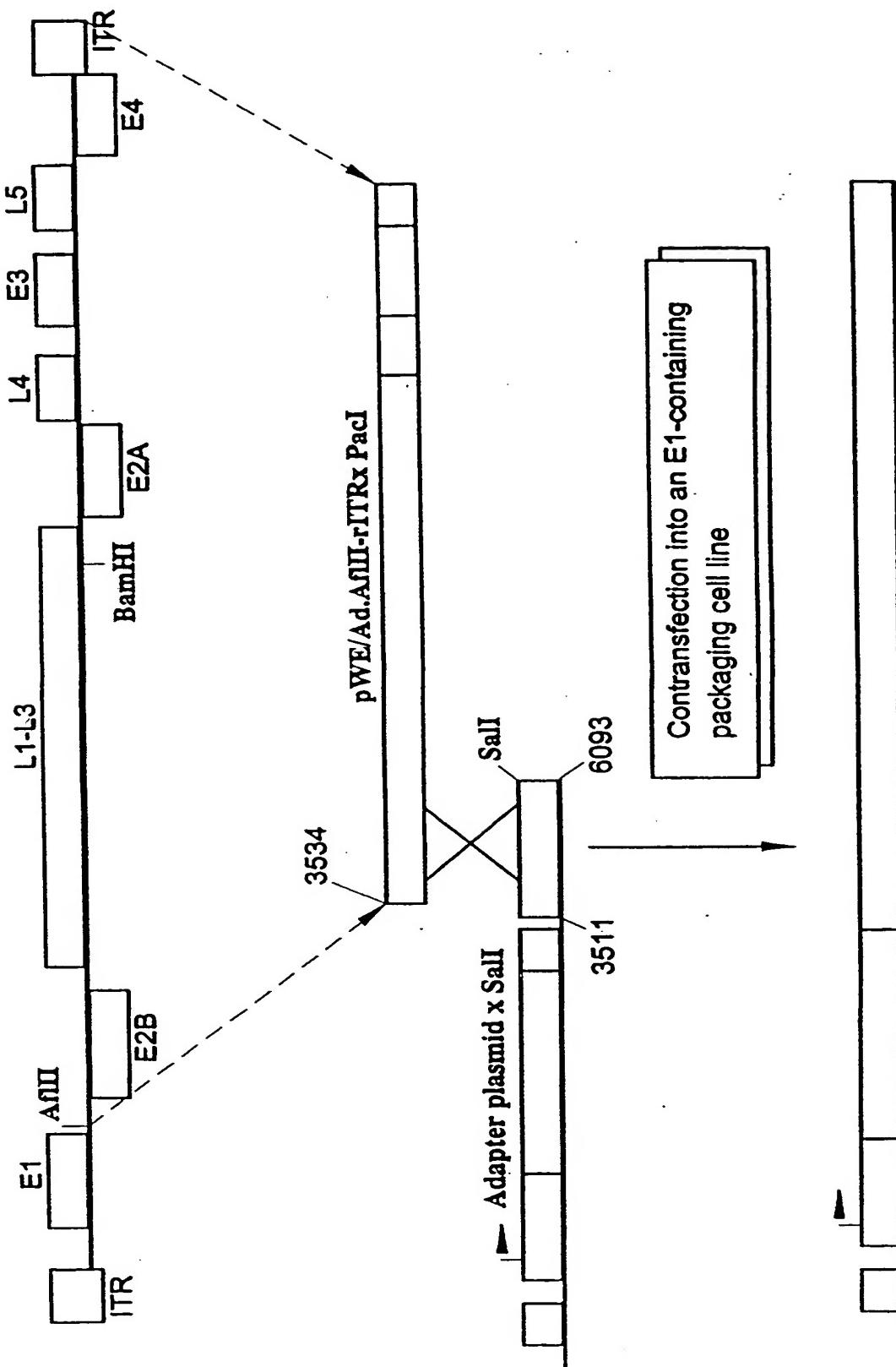


FIG. 22



Generation of recombinant adenoviruses





Minimal adenovirus vector pMV/L420H

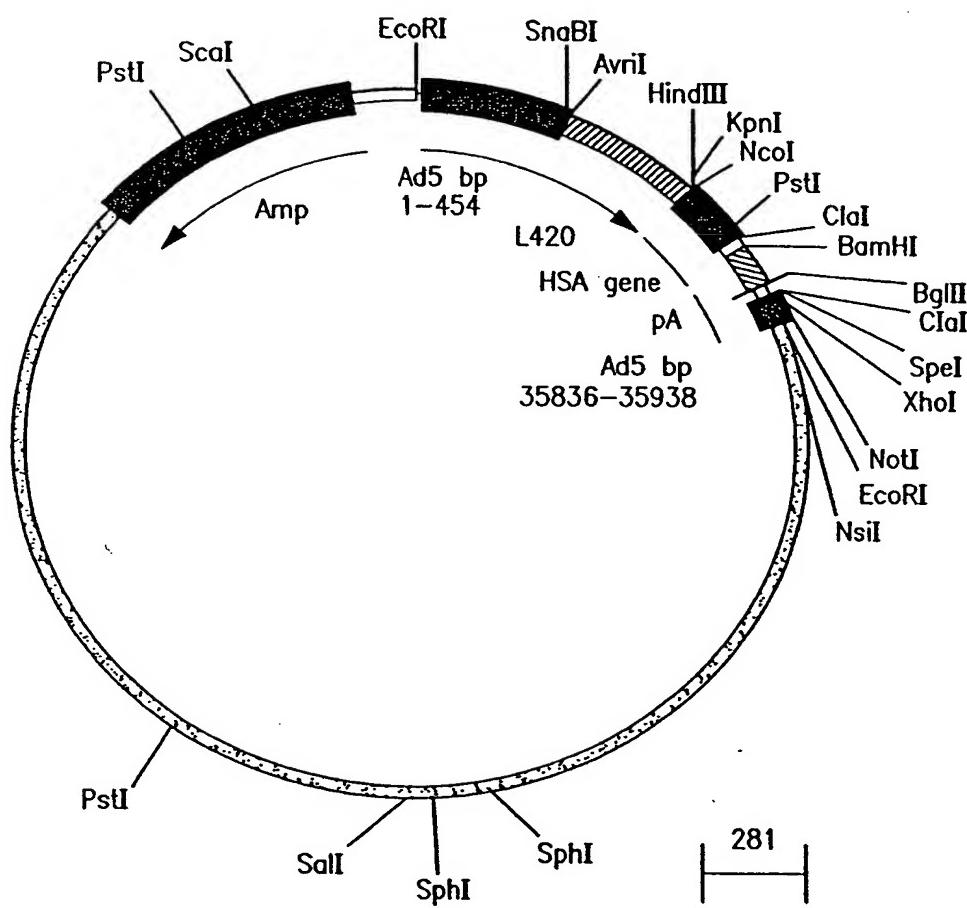
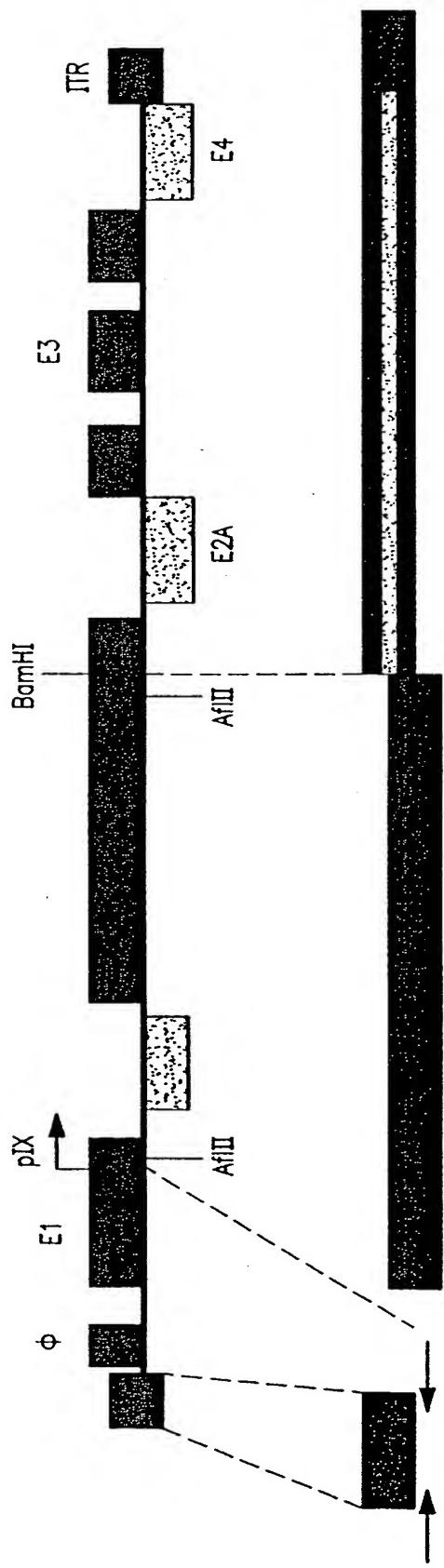


FIG. 24



Construction of pWE/Ad Δ 5'



pBr/Ad.Bam-rrTR.pac
x BamHI and PacI

pBr/Ad.Cla-Bam
x EcoRI and AfI(partial)

pcr fragment
left ITR

pAd/rrTR(Δ 5')-BamHI
xPacI, BamHI

+ pWE15.Pac x PacI

xPacI, BamHI

FIG. 25

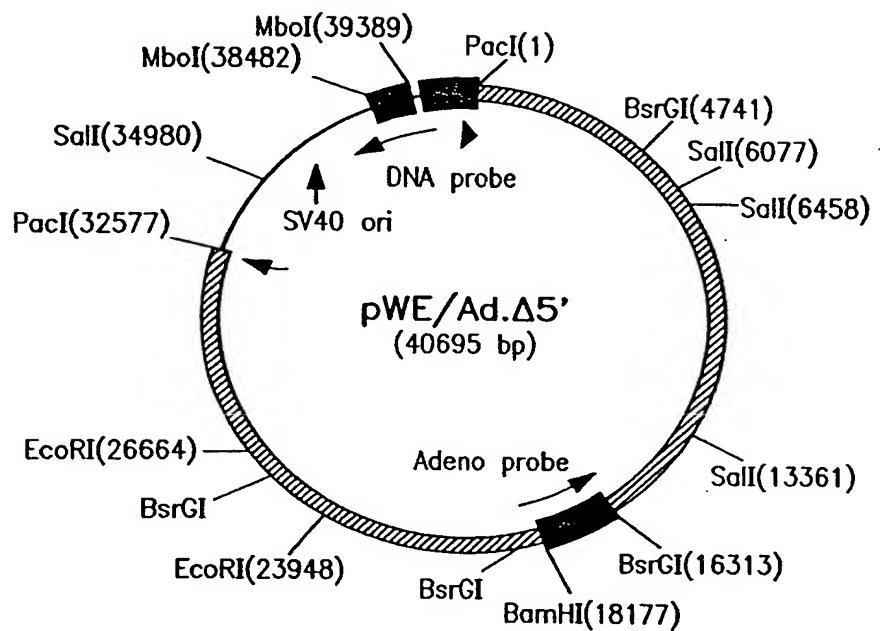


FIG. 26A

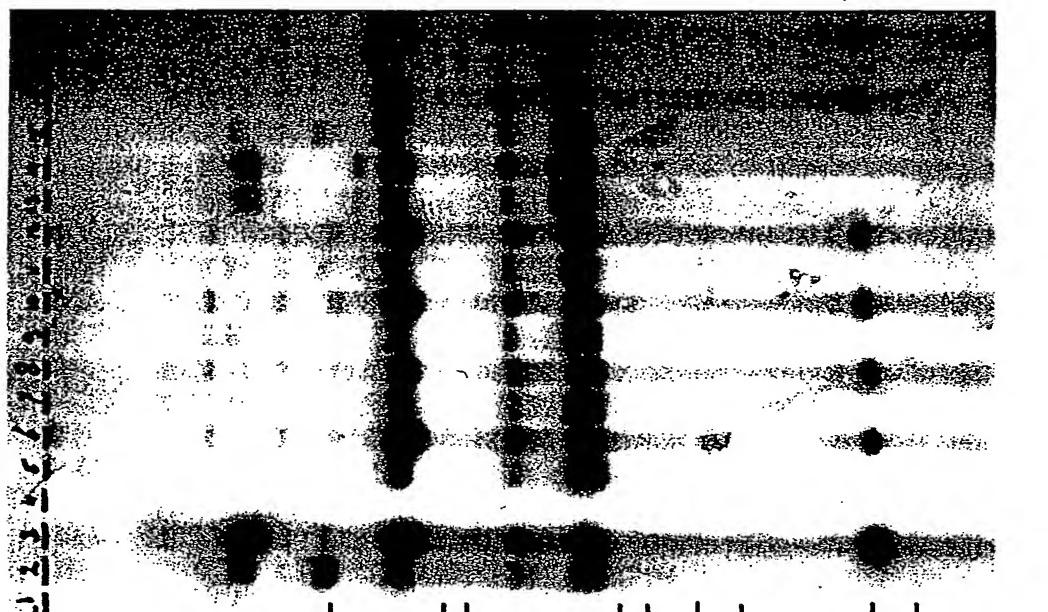


FIG. 26C

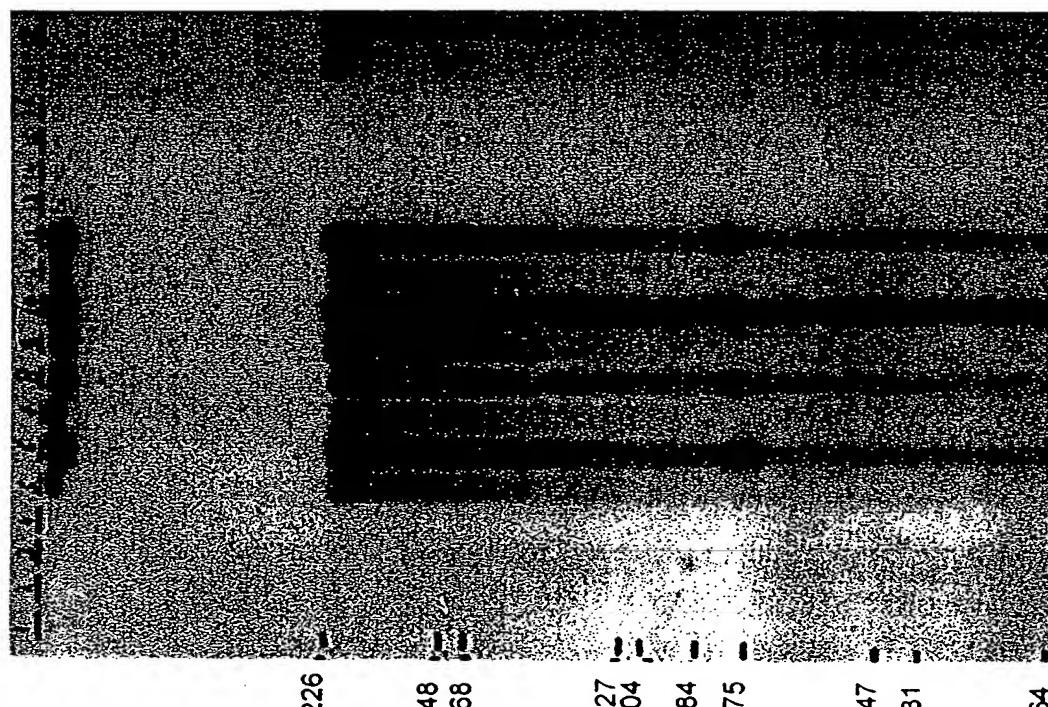
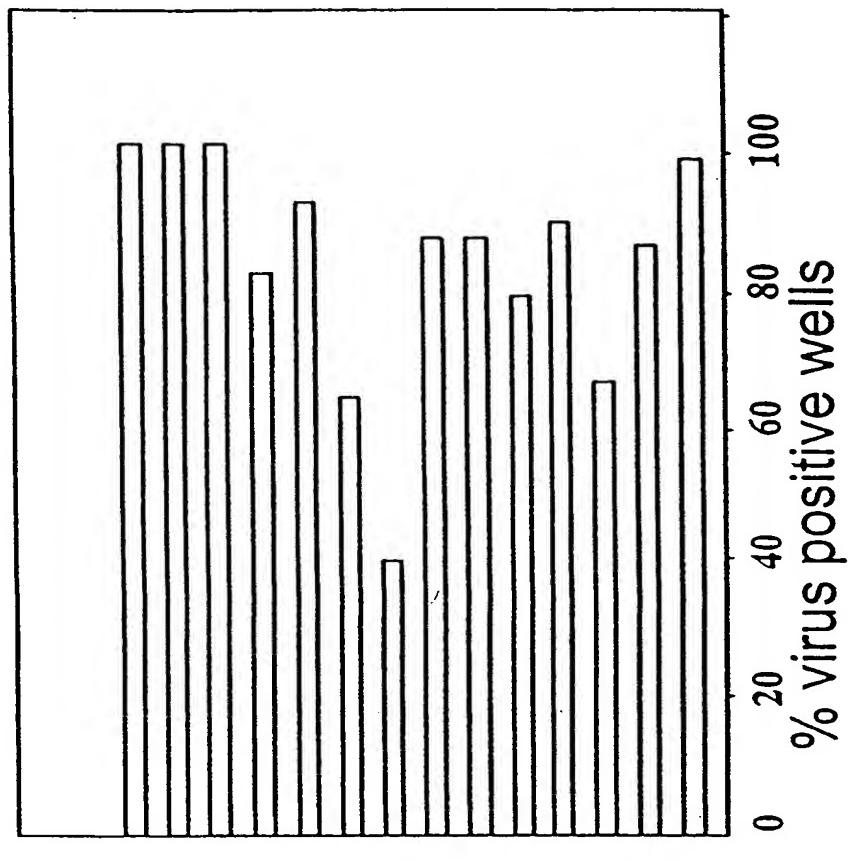


FIG. 26B



CDNA insert

Average percentage CPE efficiency: 86 %

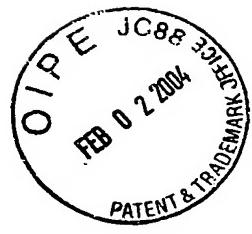
FIG. 27

Gene Insert kb

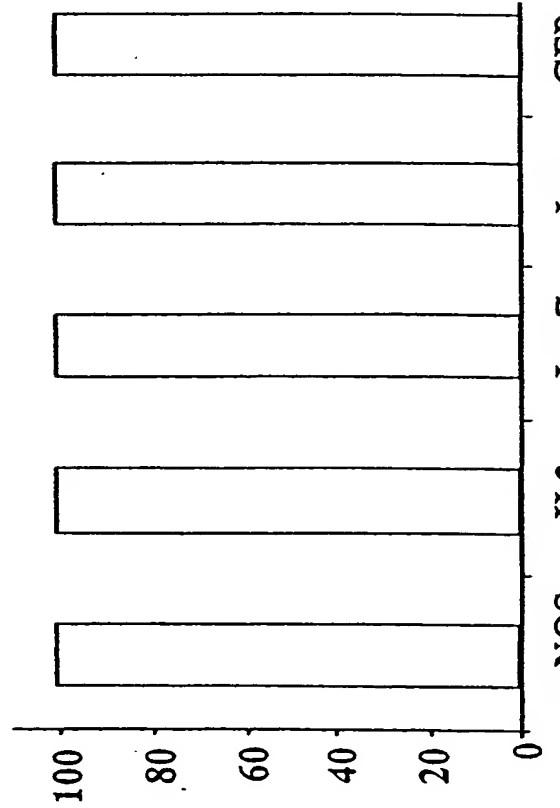
Gene	Insert kb	Average titer $0.8 \pm 0.7 \times 10^9$ pfu/ml
cenOS	3.6	
hTERT	3.5	
hTERT D712A	3.5	
lacZ	3.2	
hCAT1	2.2	
GLVR2	2.0	
Luc	1.7	
SOD3	1.4	
MAX1	.550	
hVEGF121	.511	
hIL3	.434	
UBC9	.412	
ANG1-7	.104	

FIG. 28





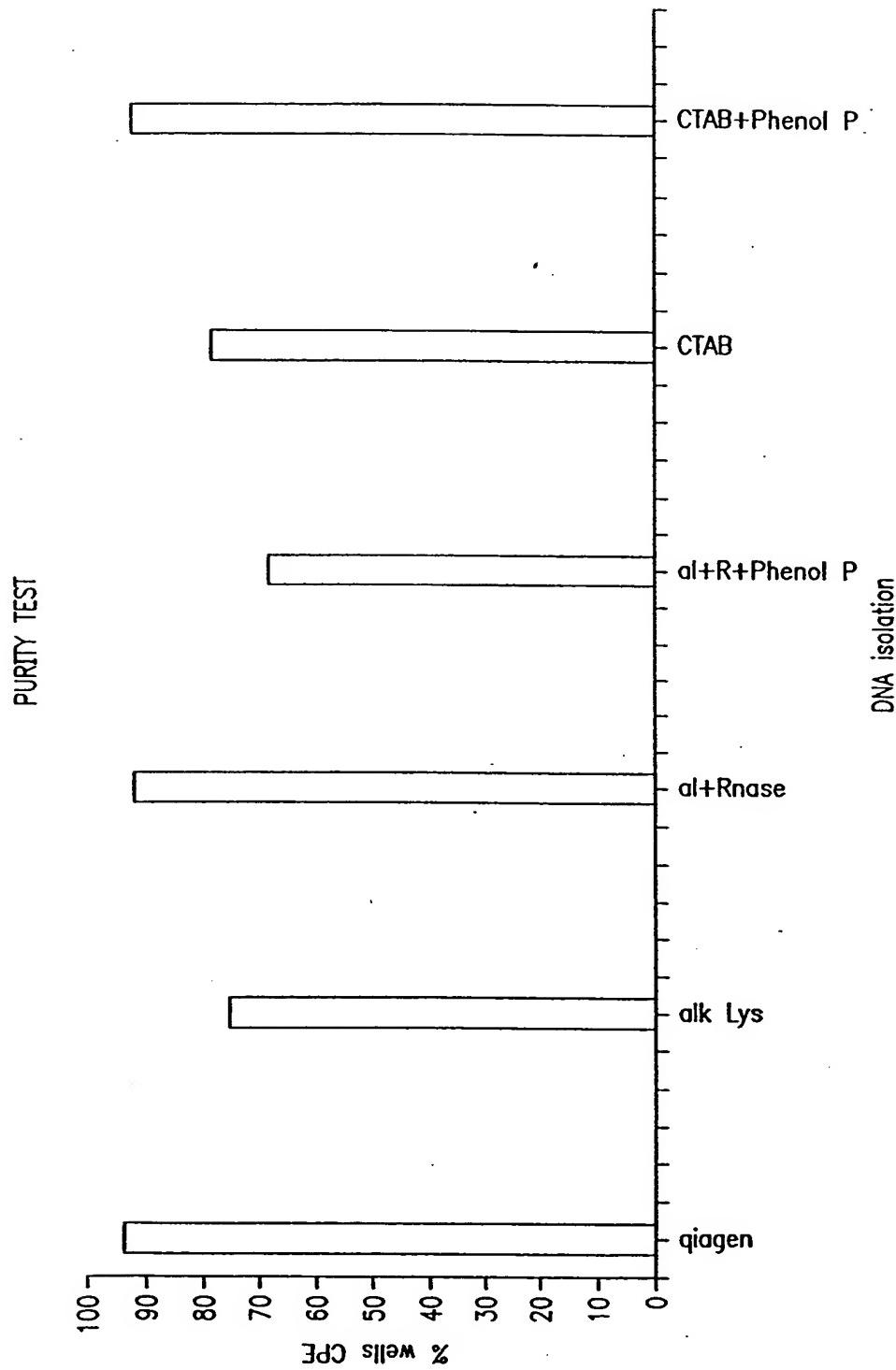
% wells producing functional virus



Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

FIG. 29



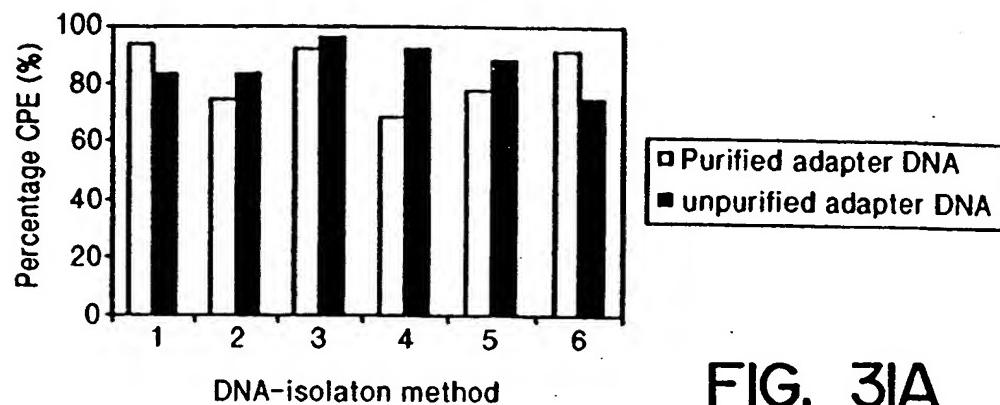


FIG. 3IA

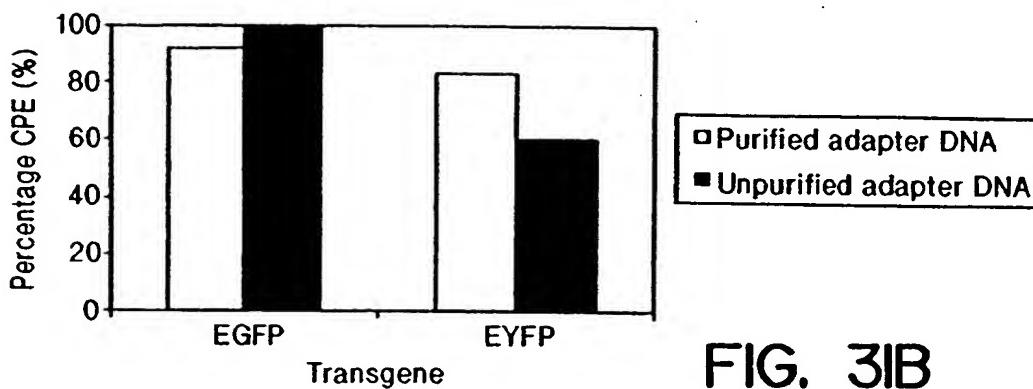


FIG. 3IB

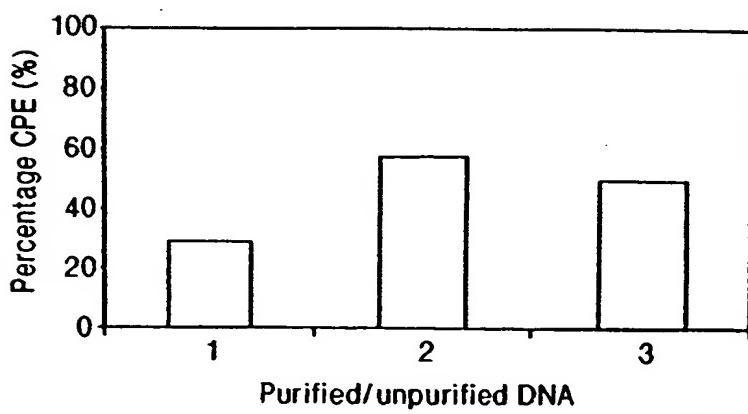


FIG. 3IC

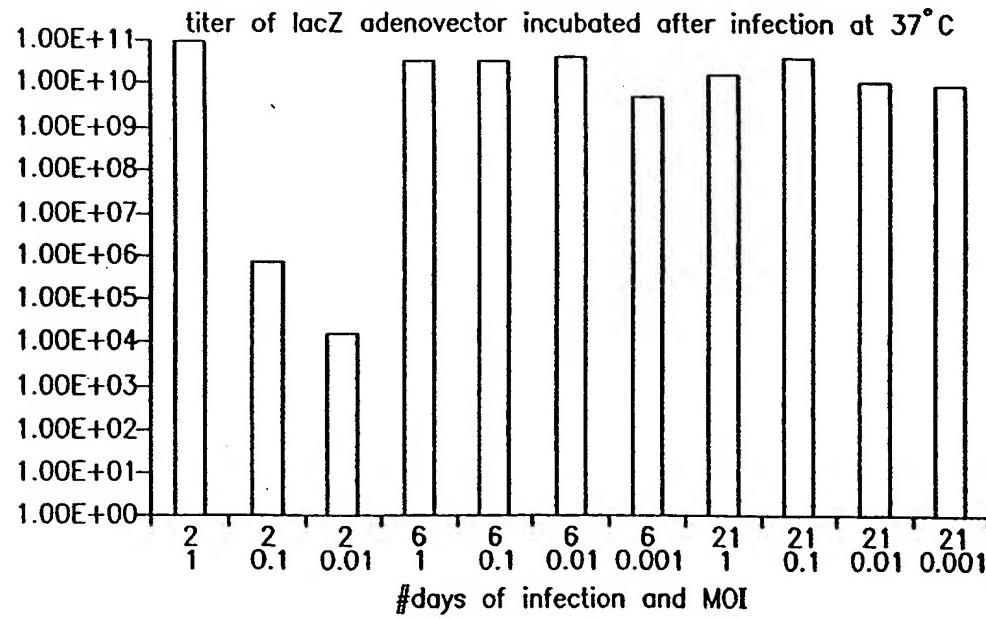
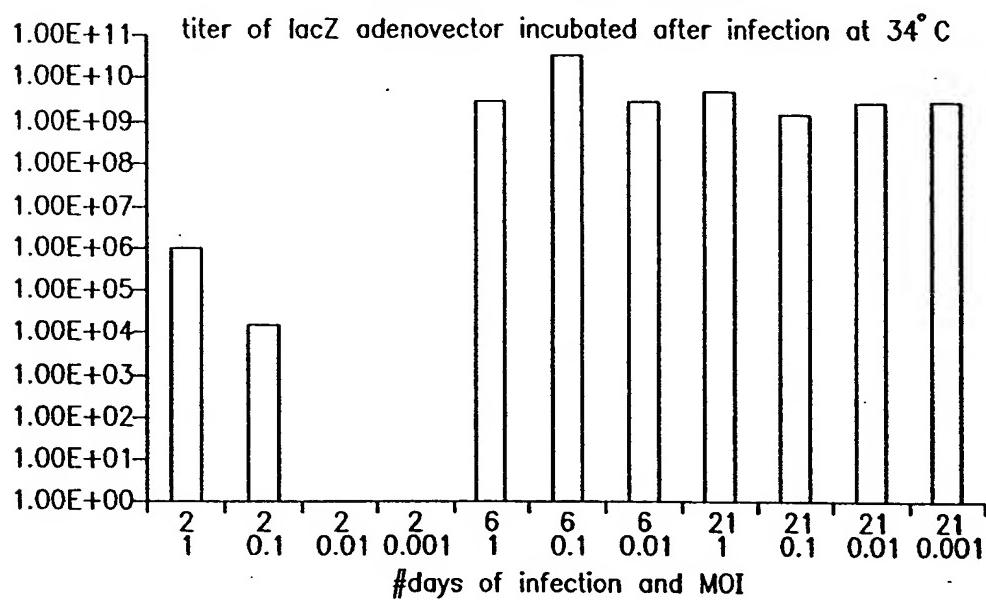
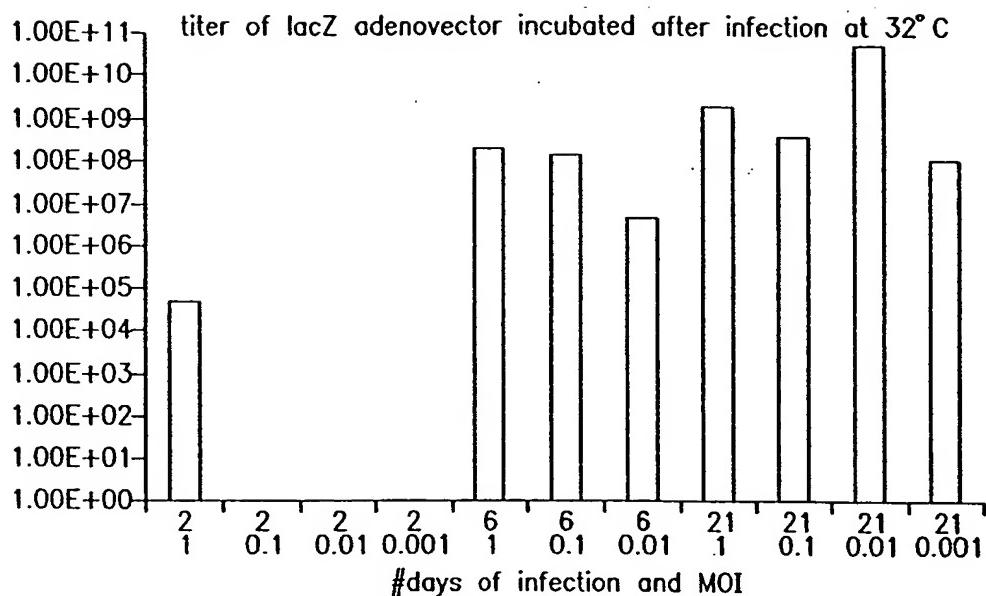


FIG. 32

FEB 02 2004
PATENT & TRADEMARK OFFICE
U.S.

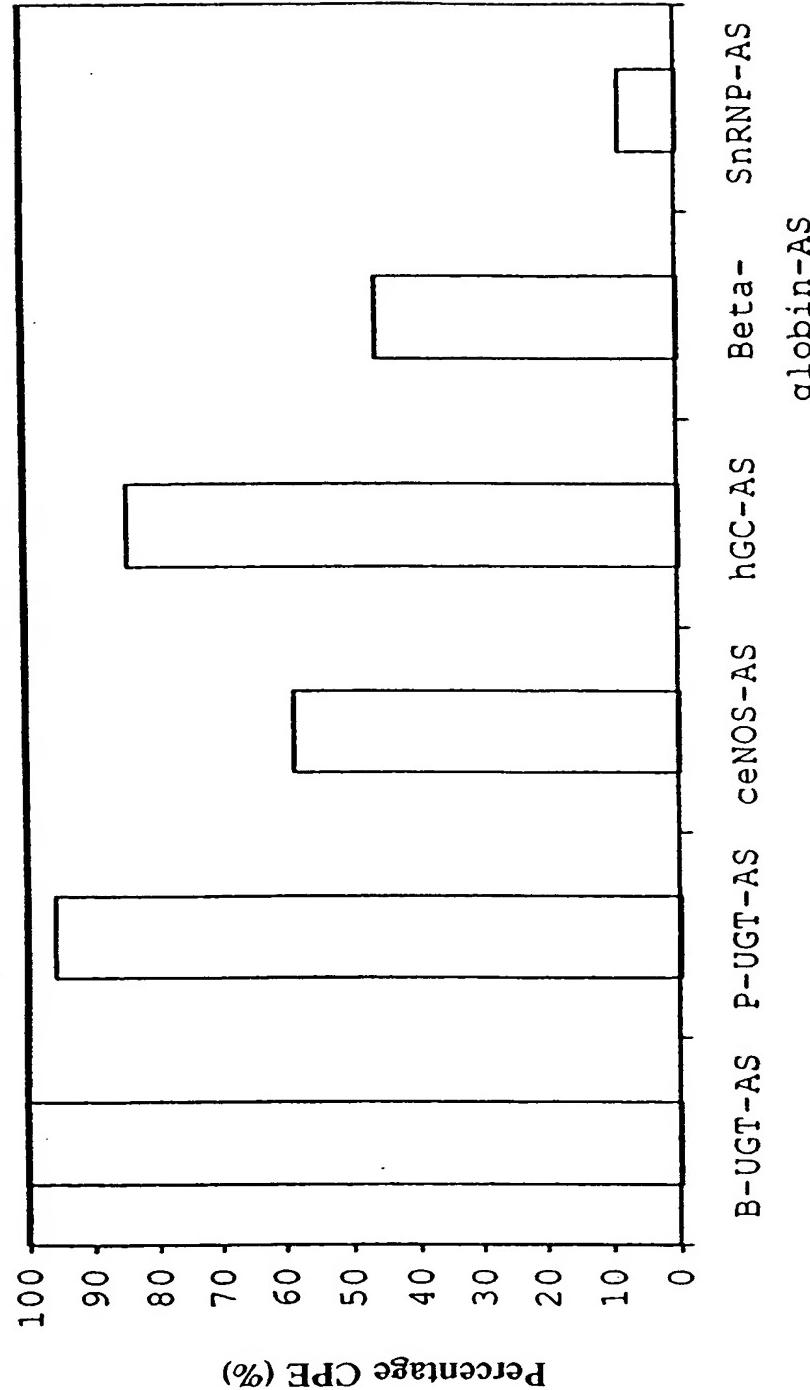


FIG. 33

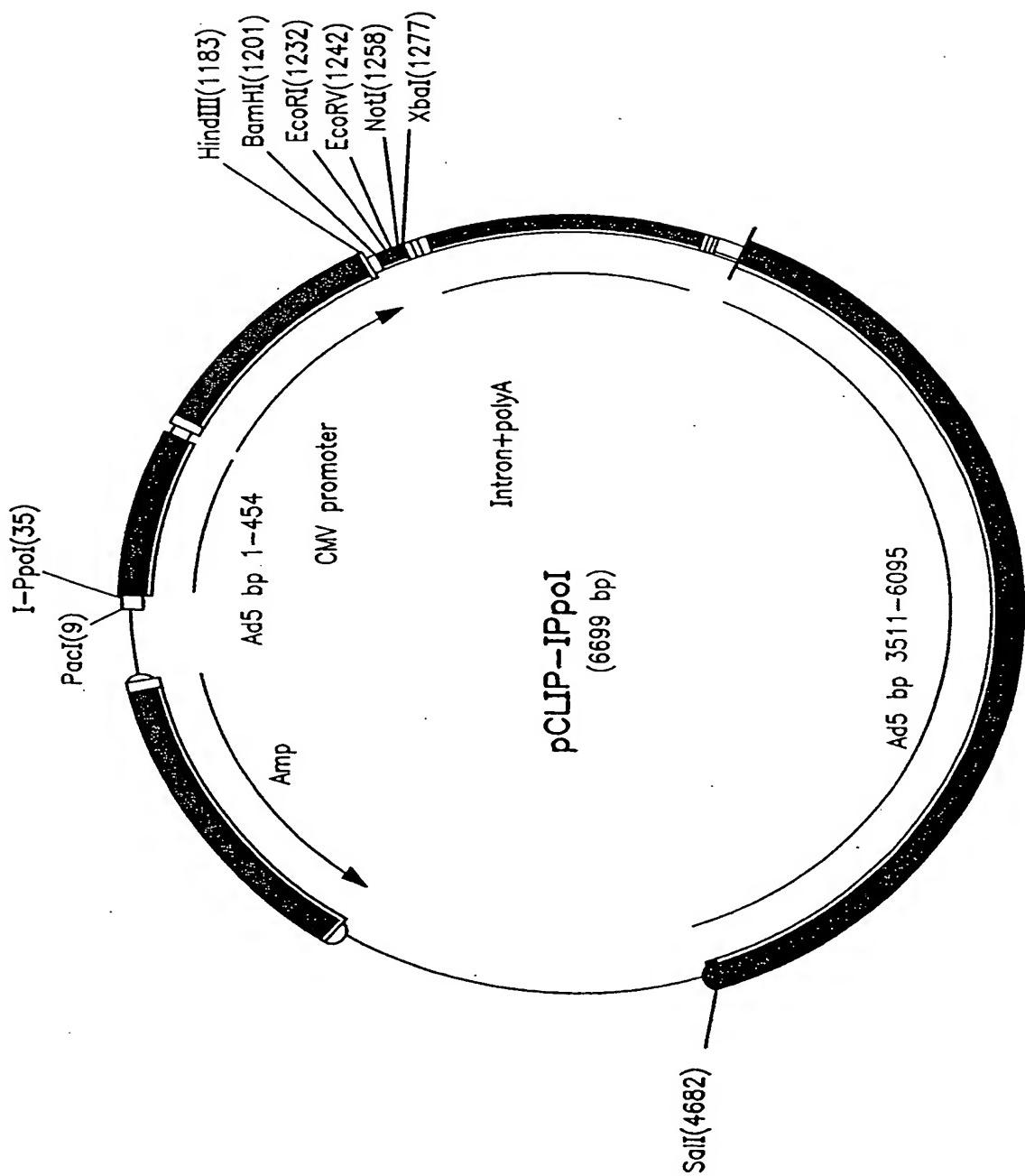


FIG. 34A

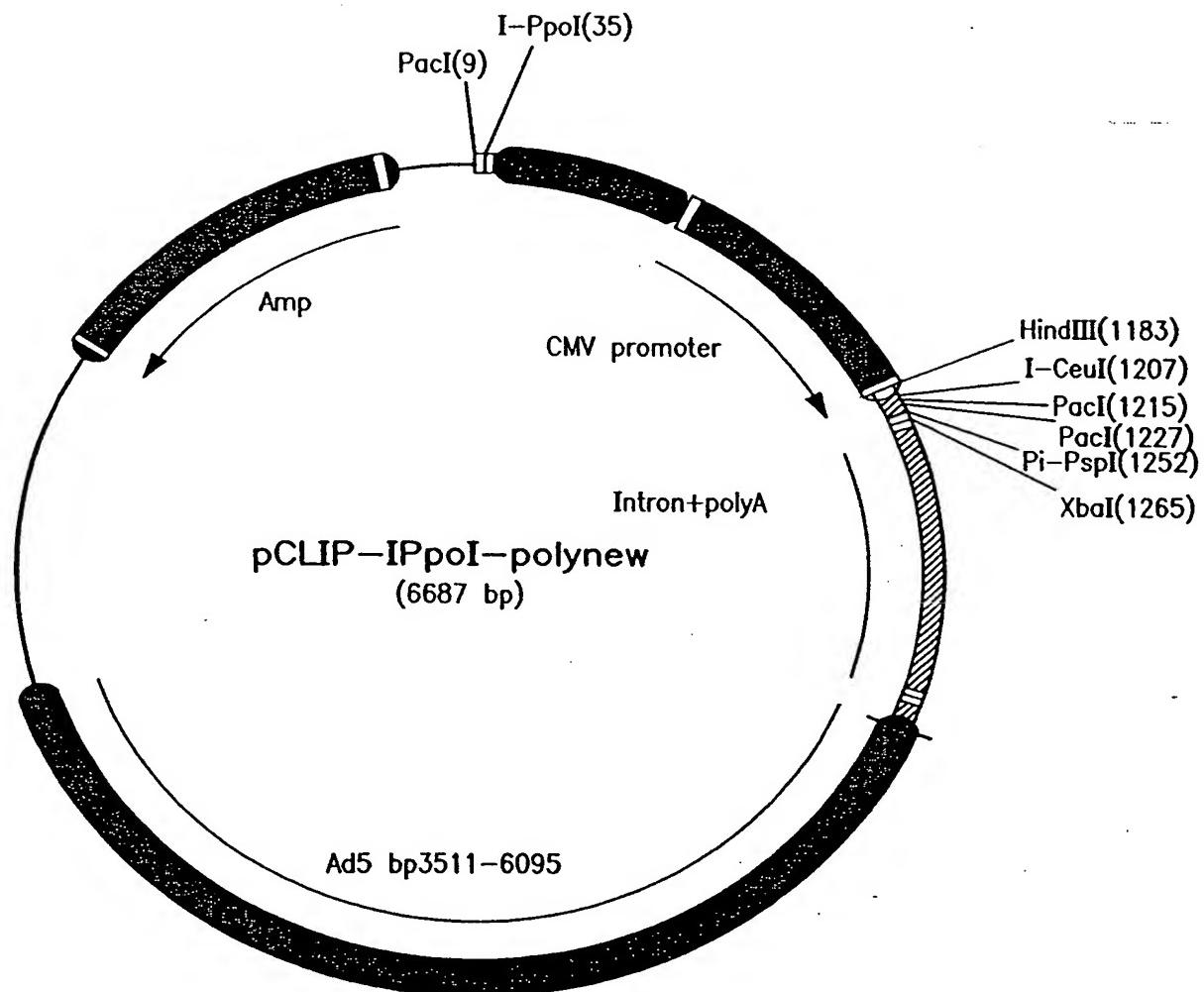


FIG. 34B

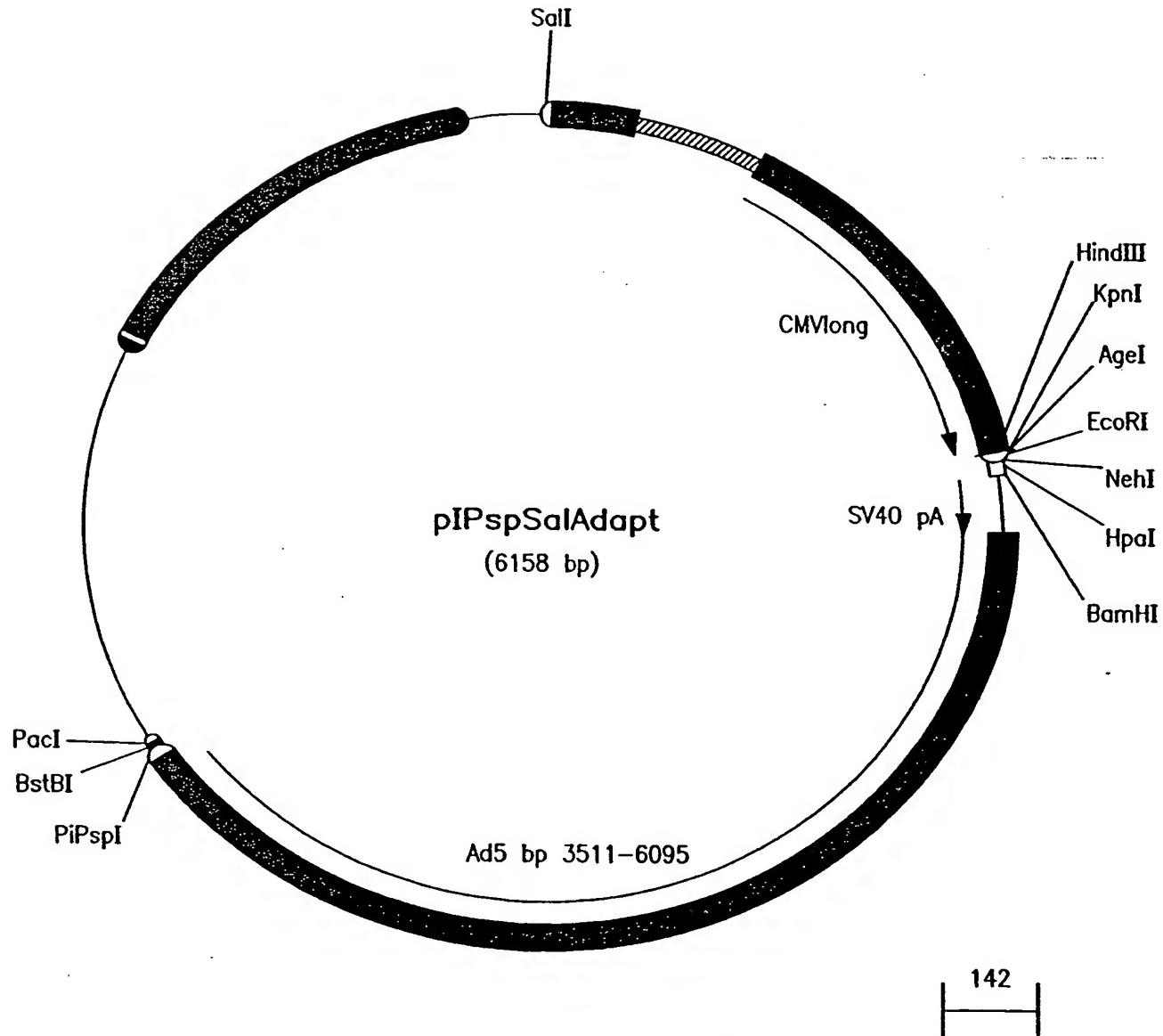


FIG. 34C

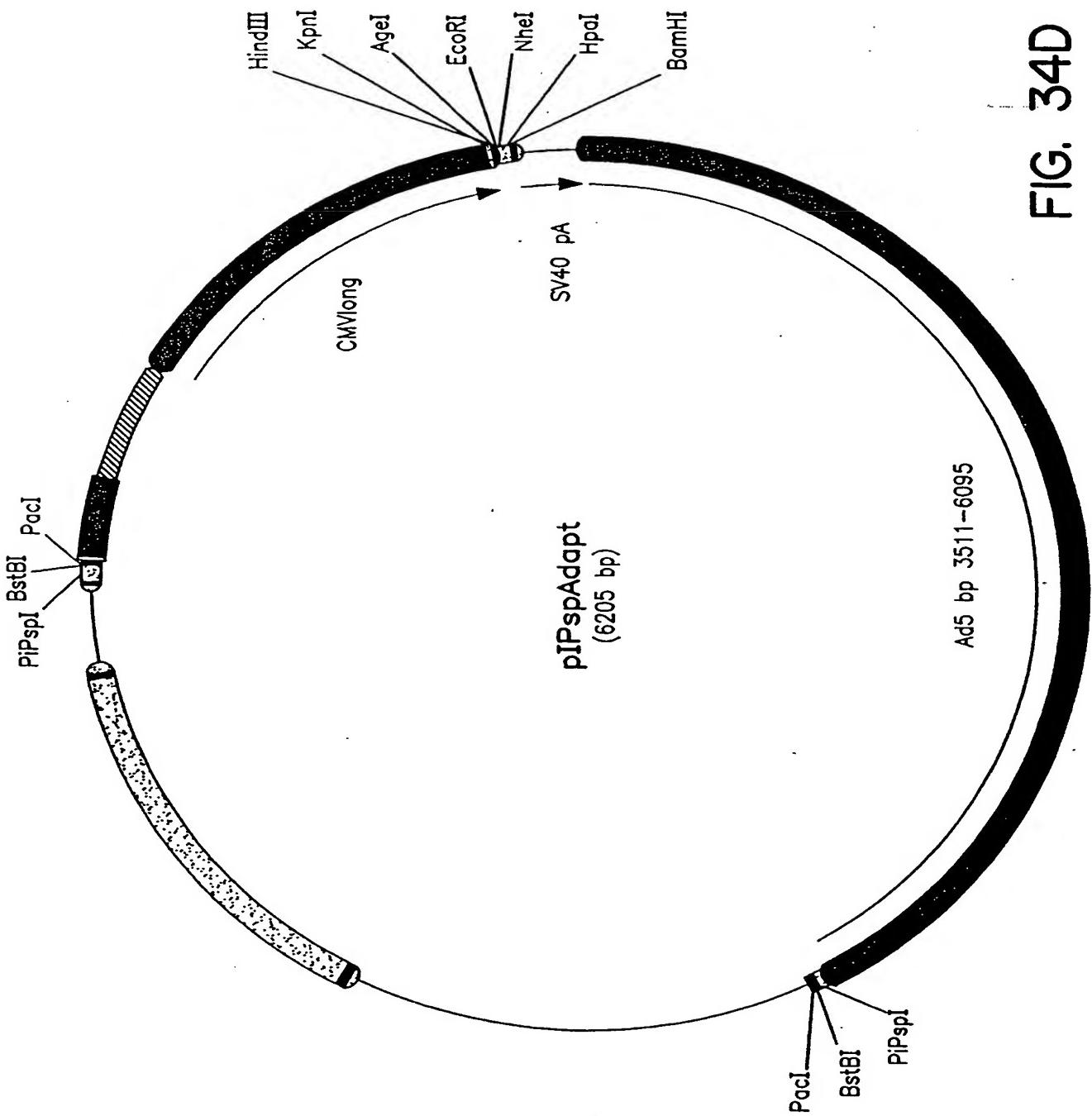


FIG. 34D

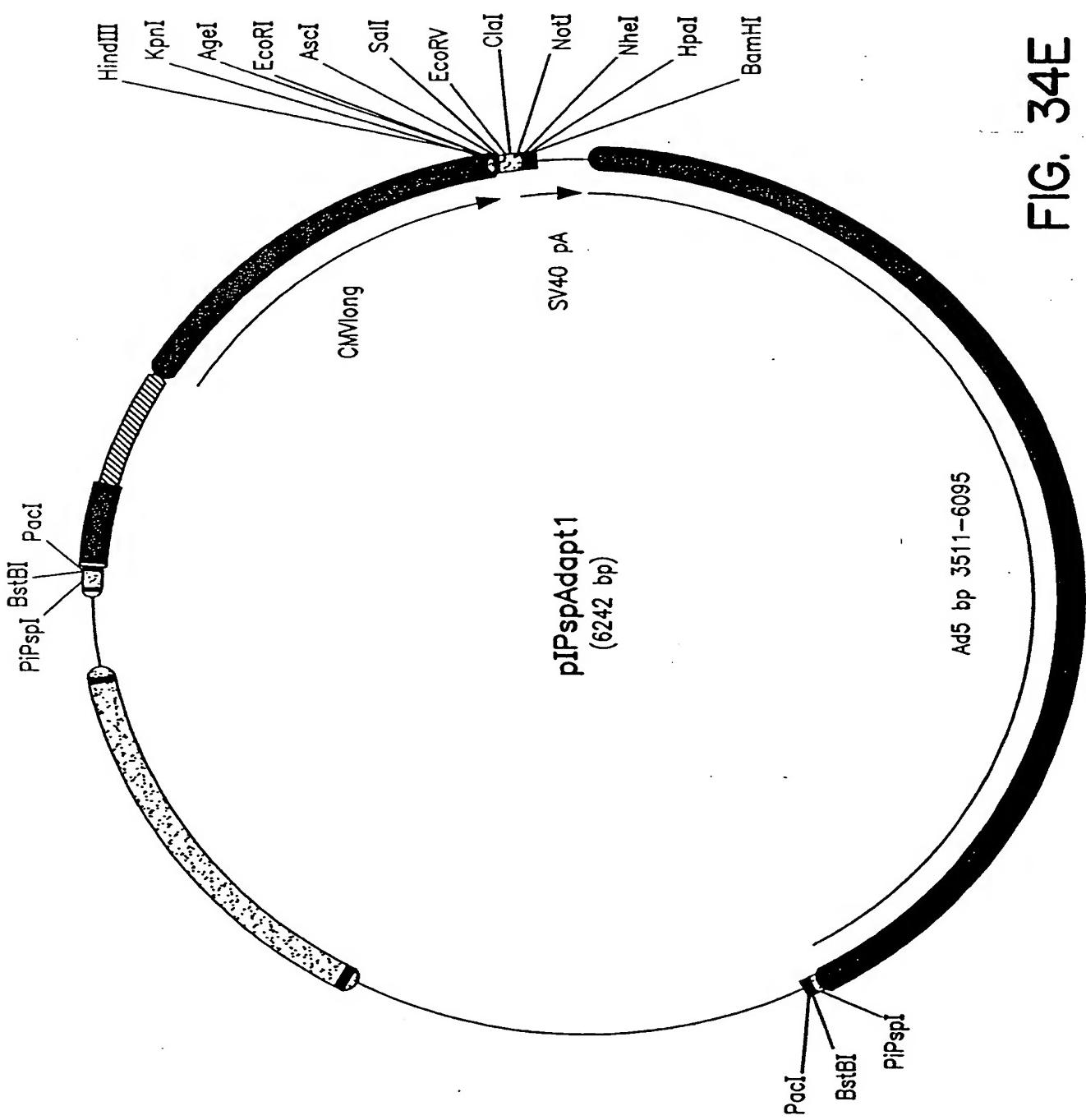


FIG. 34E

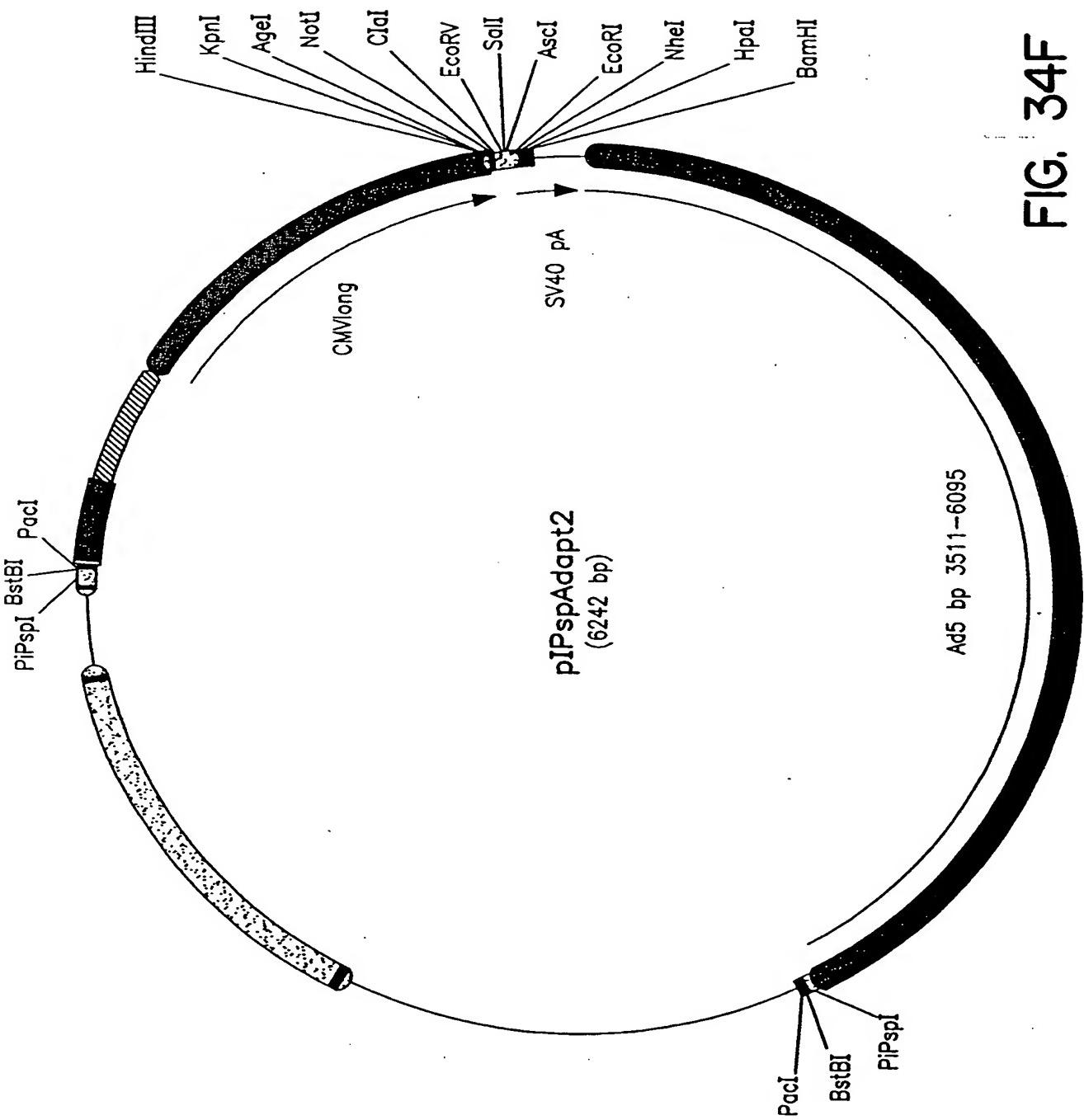


FIG. 34F

Ad5 pp 3511-6095

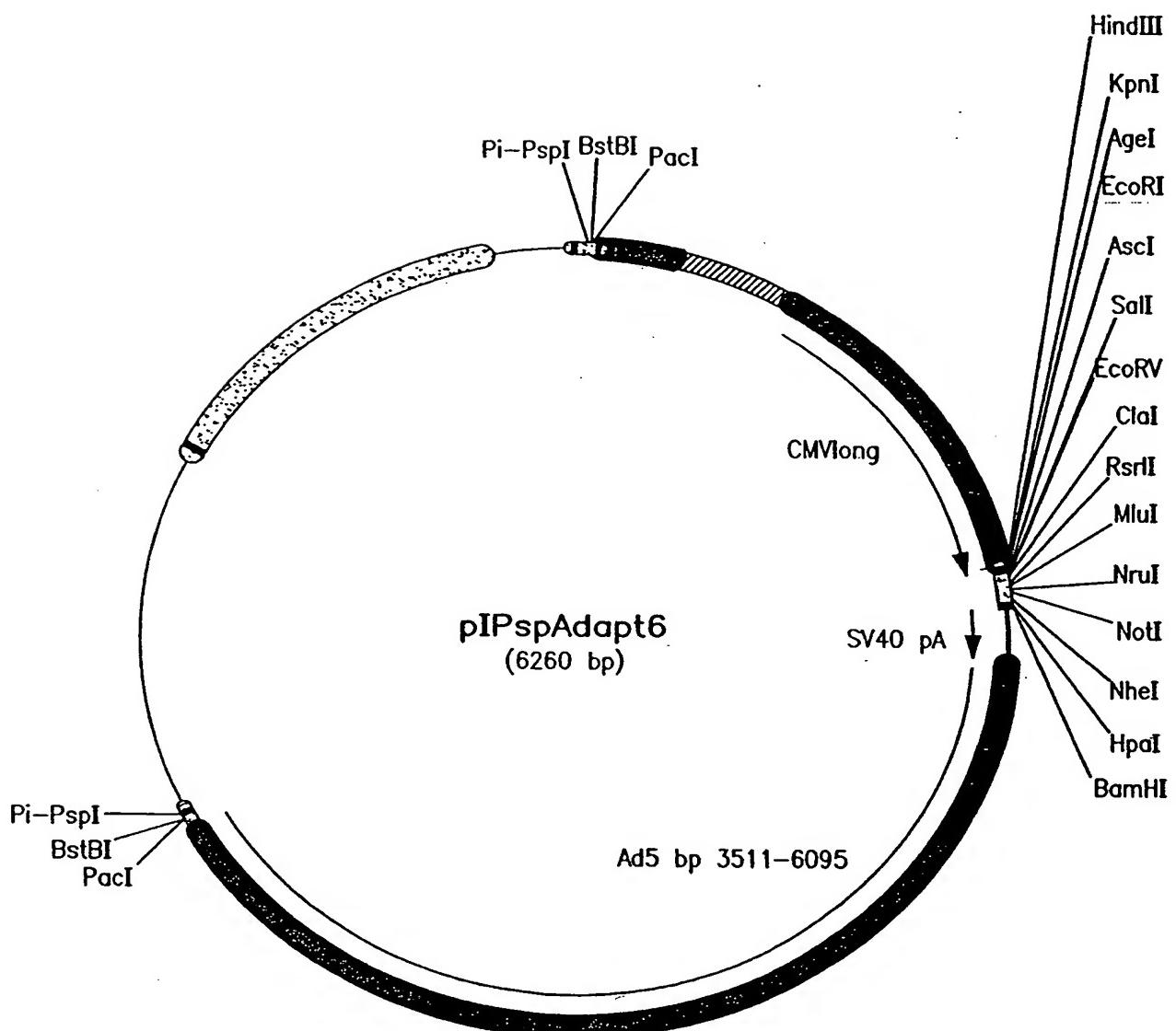


FIG. 34G

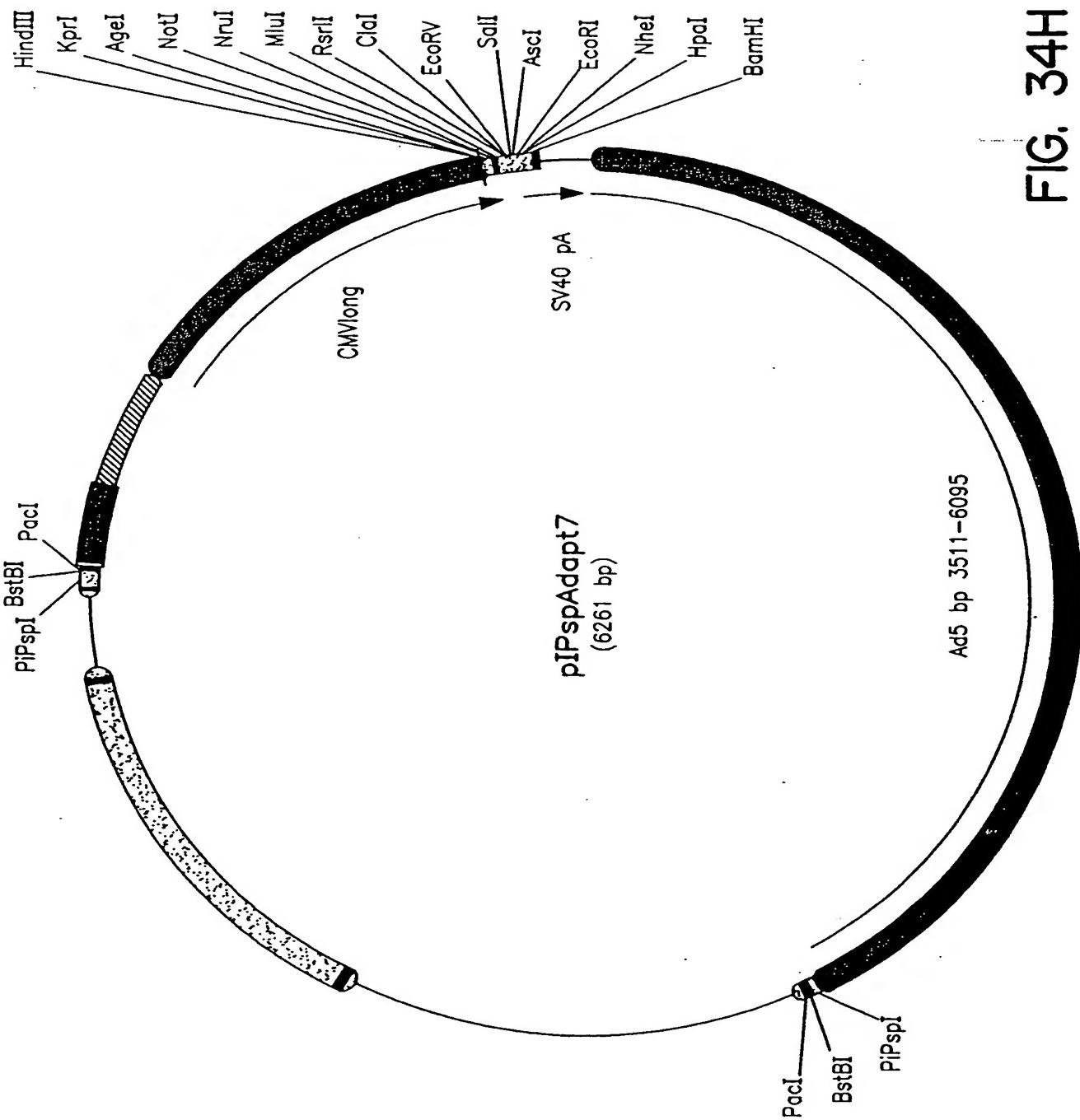


FIG. 34H

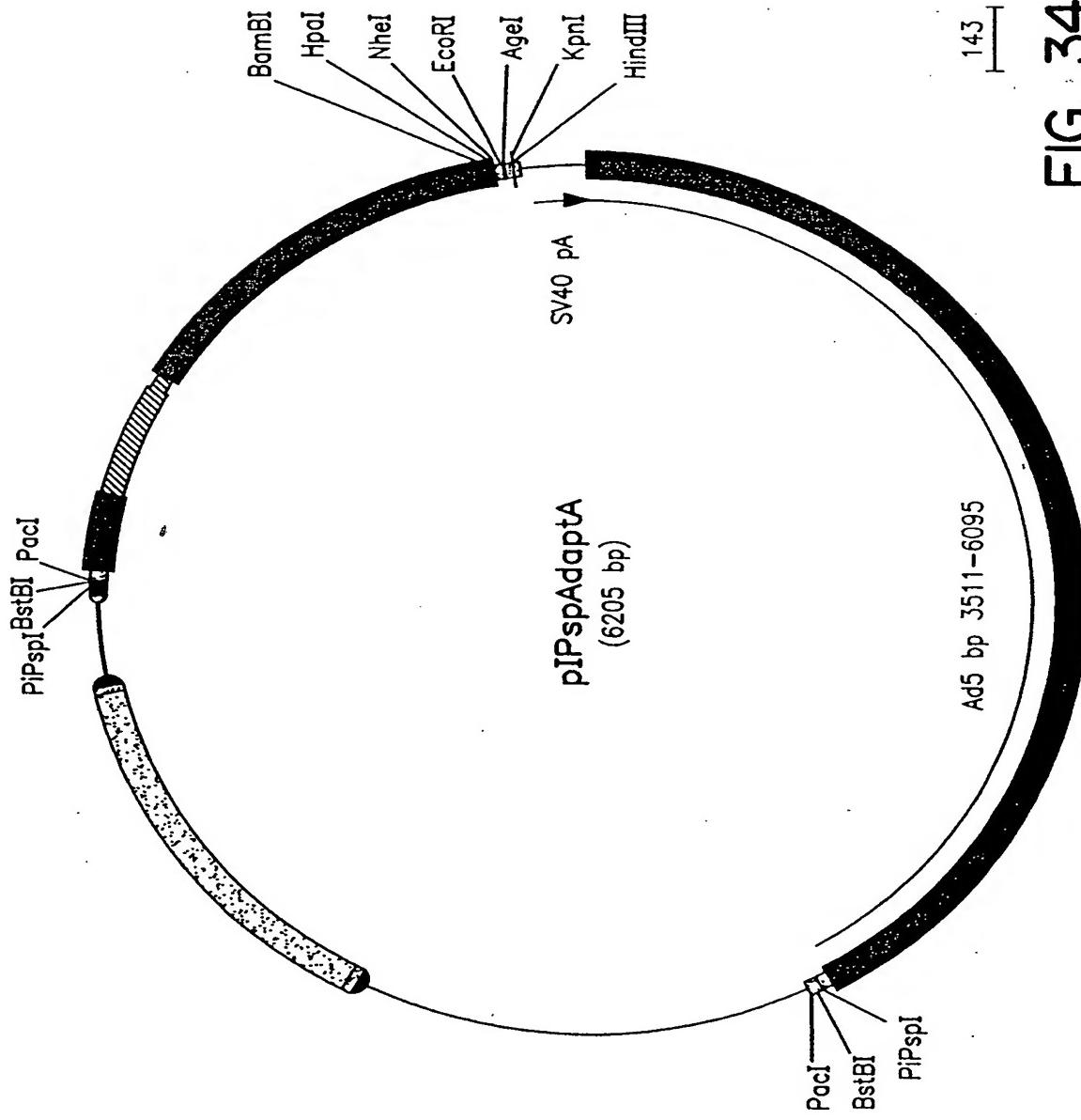


FIG. 34 |

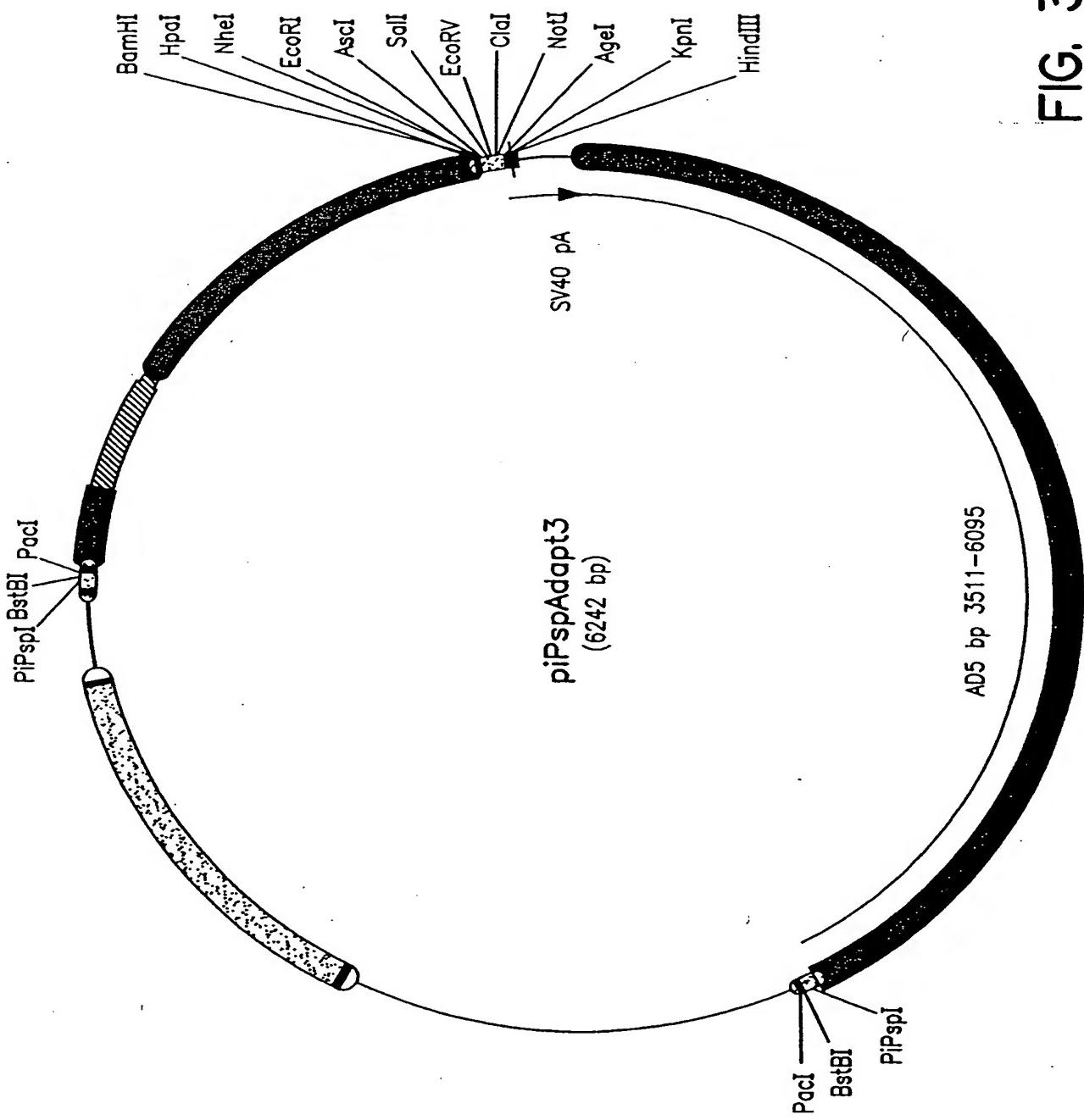


FIG. 34J

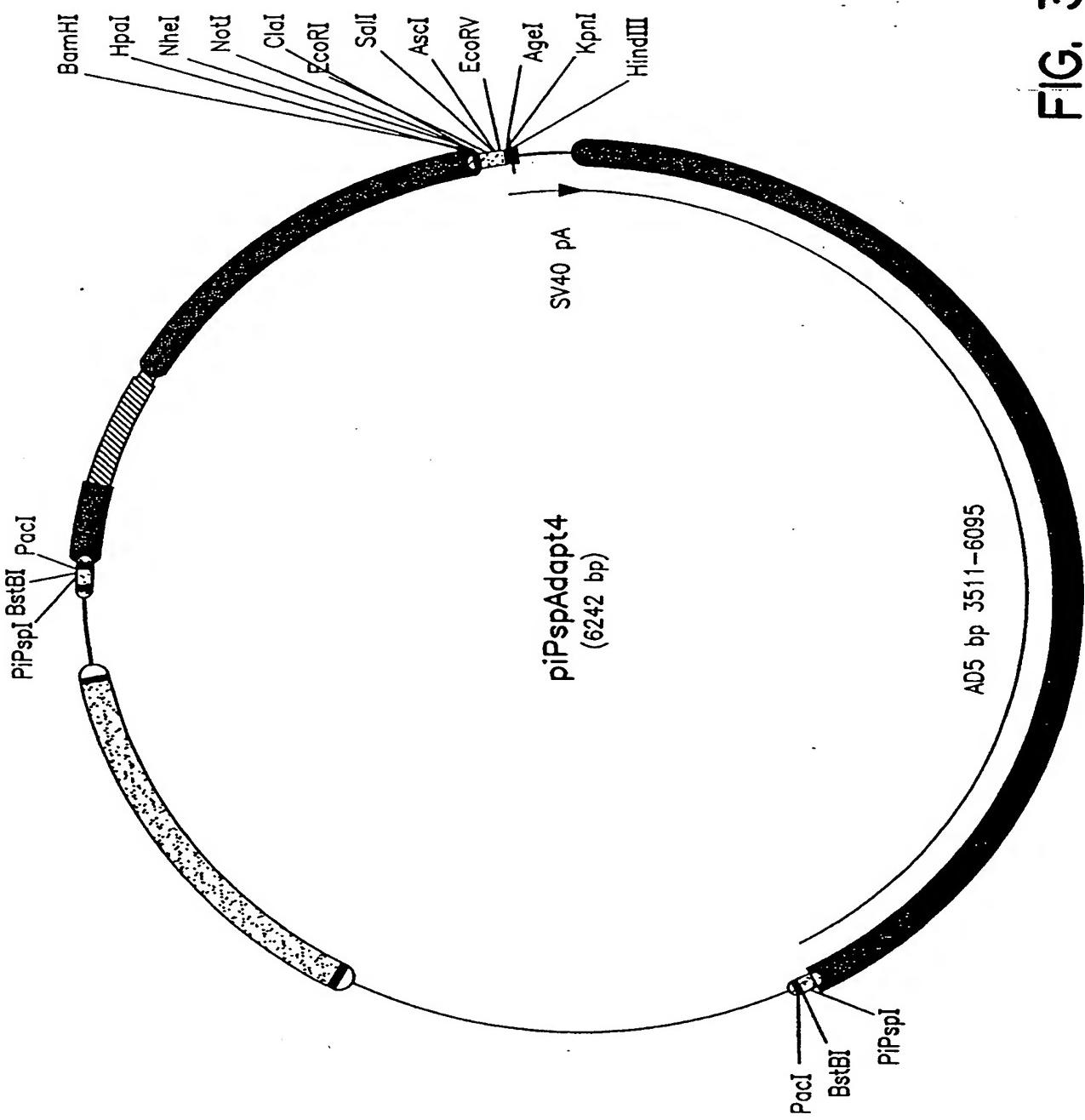


FIG. 34K

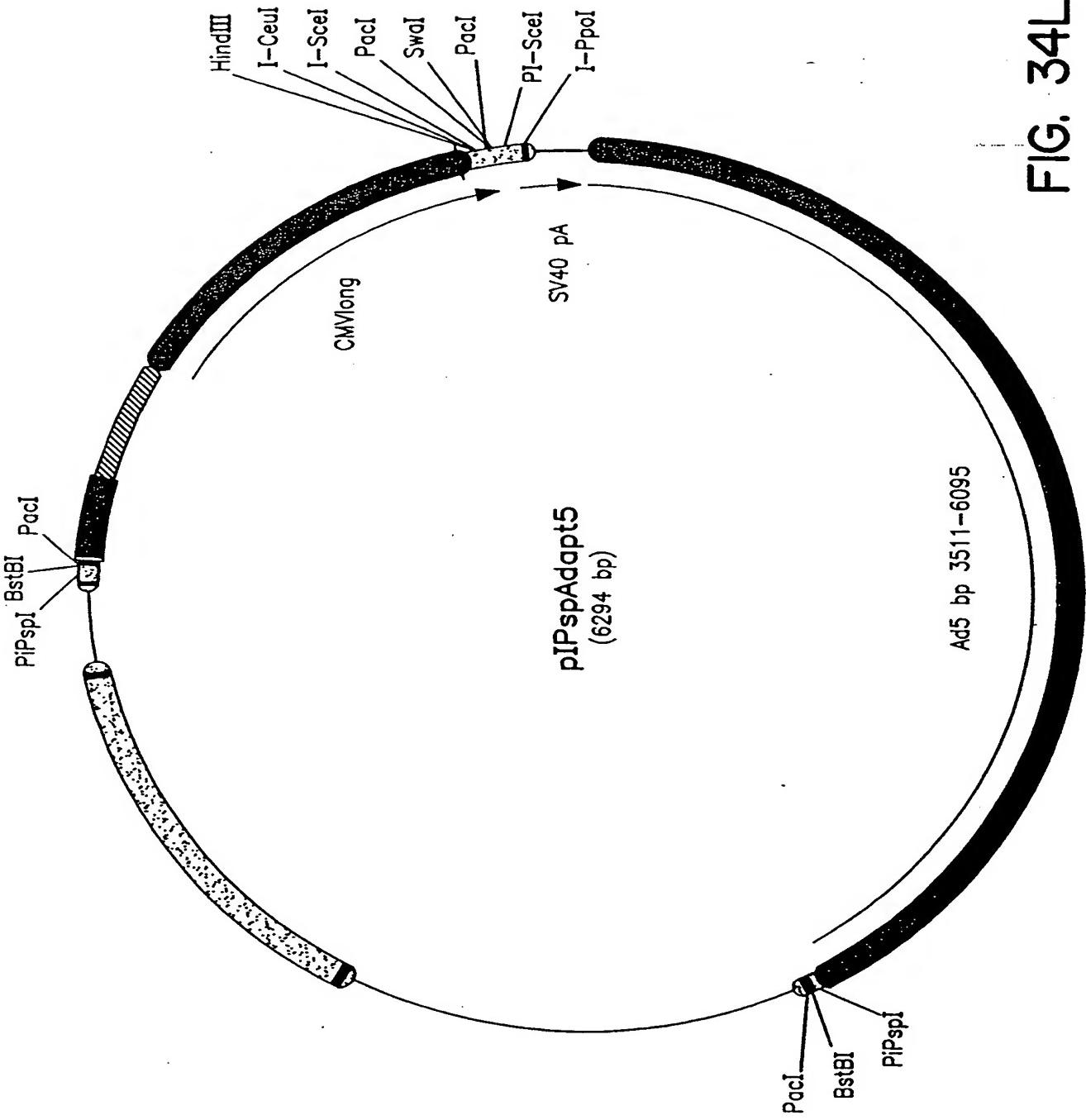


FIG. 34L

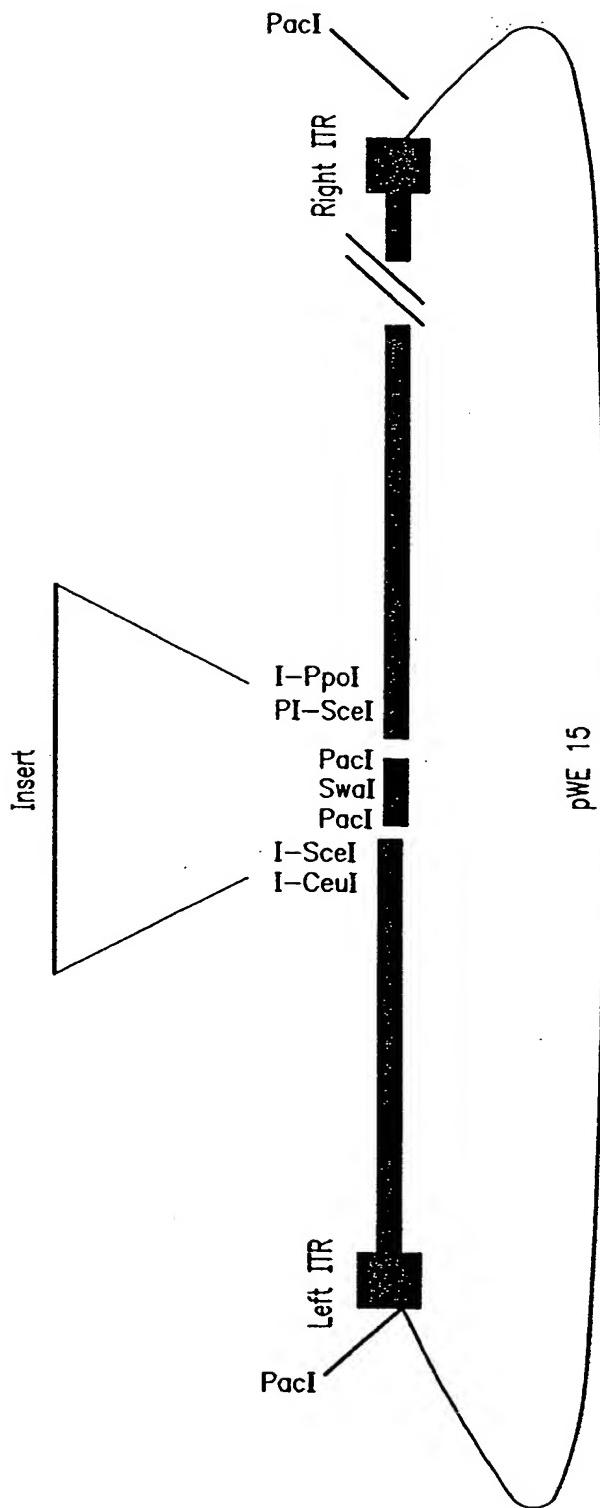


FIG. 34M



Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP-LacZ and the adapter plasmid pIPspAdapt2.

Transfection of pIPspAdapt2 to PER.C6/E2A

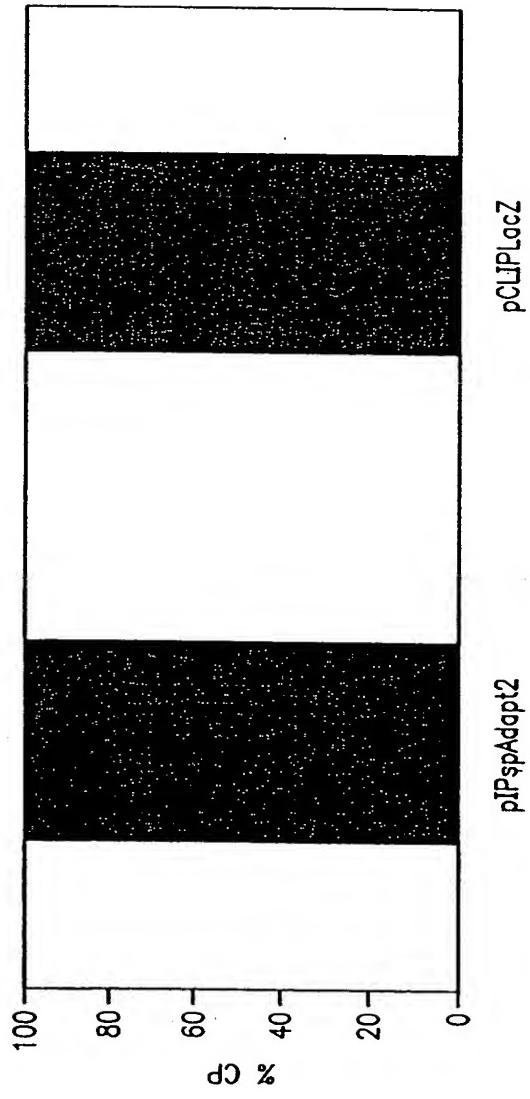


FIG. 34N

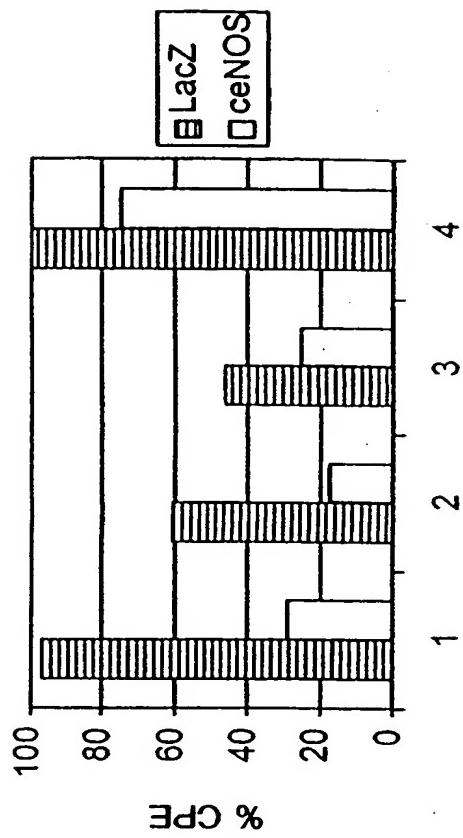


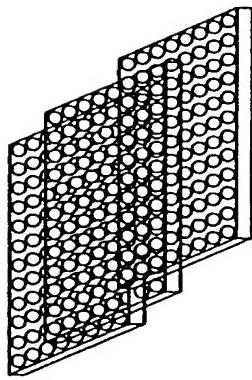
FIG. 35



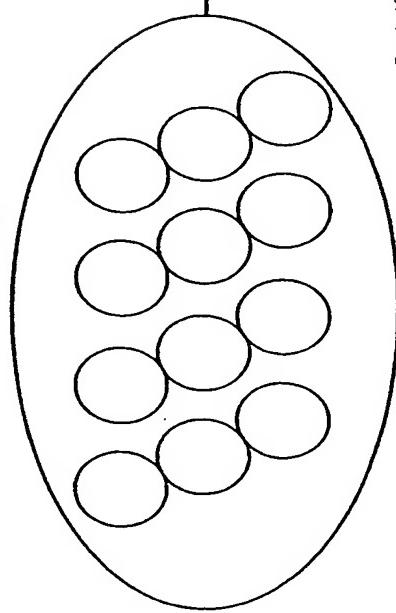
Construction total Adeno cDNA Library (1)

Cells/tissue → mRNA isolation → cDNA →

E.coli transformation



transfer colonies



Isolation of adapter plasmids
with c DNA

Linearize
adapters

FIG. 36A



Construction total Adeno cDNA Library (II)

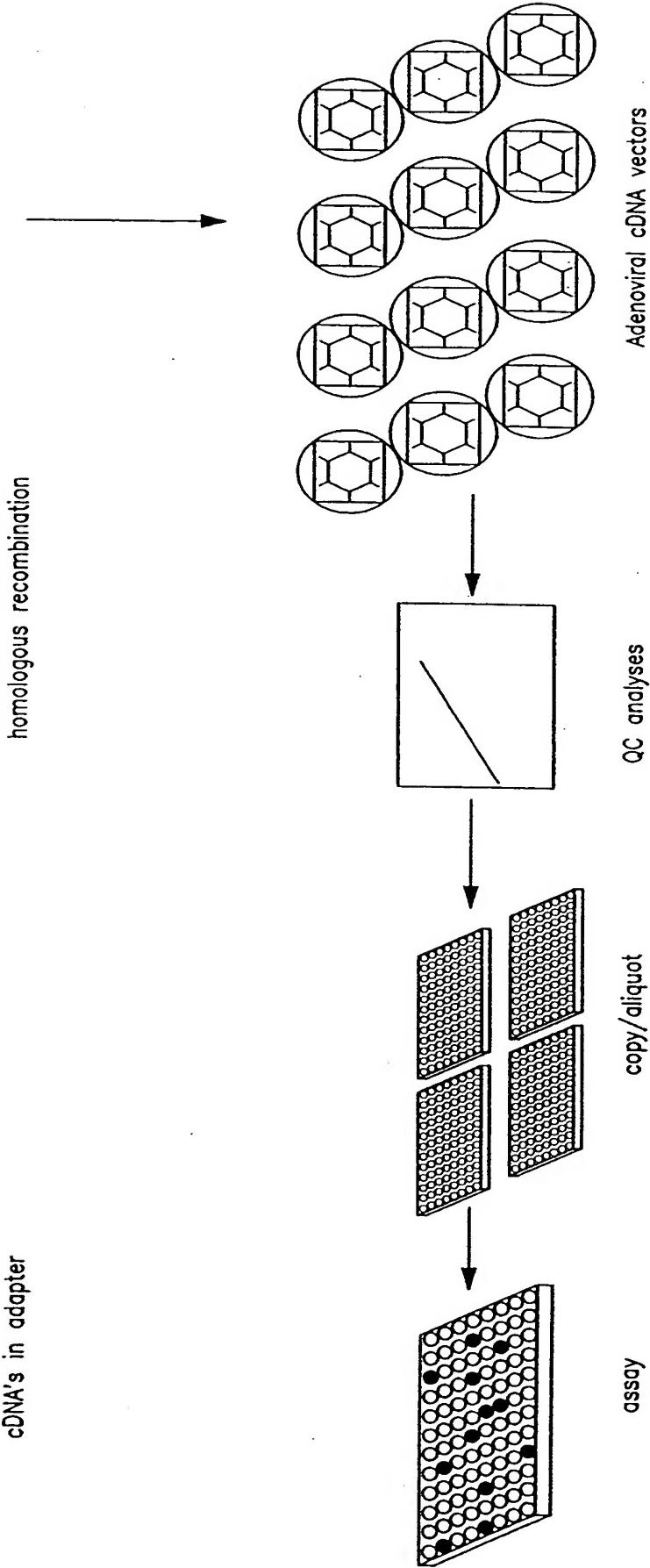
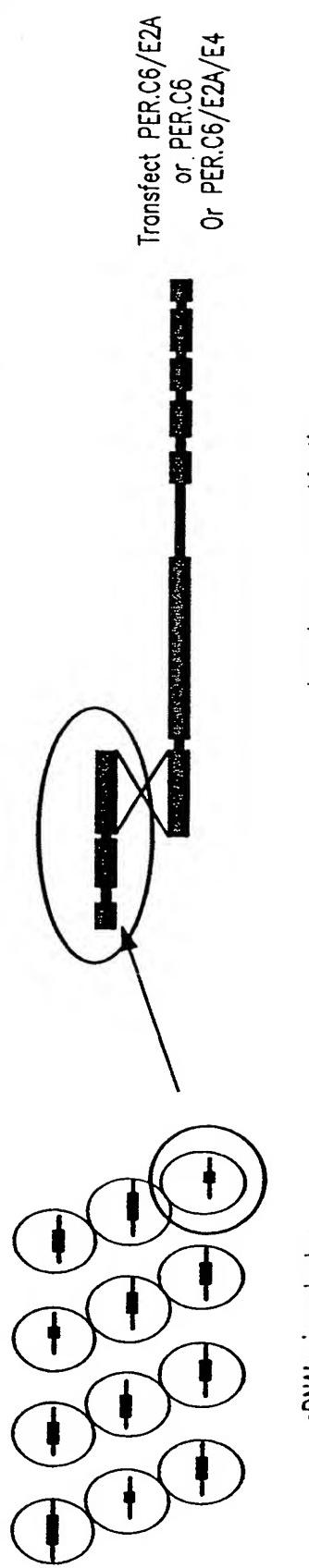


FIG. 36B



EXAMPLE 21. 384 WELL PLATE IN PROGRESS

Co-transfections on 384 well plates



FIG. 37A

Co-transfections on 96 well plates
(control plate)

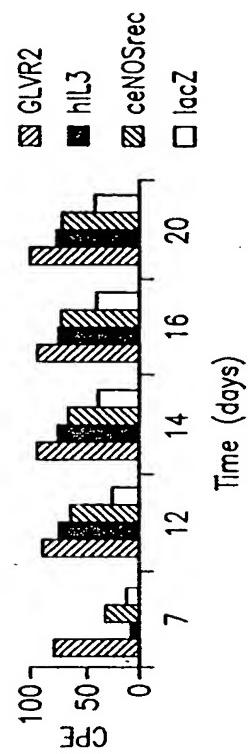


FIG. 37B

Co-transfections on 384 well plates

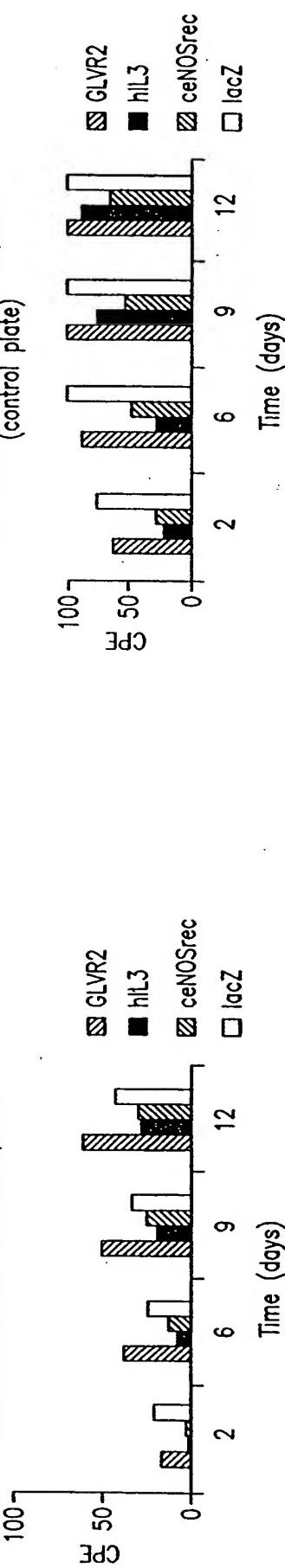


FIG. 37C

Co-transfections on 96 well plates
(control plate)

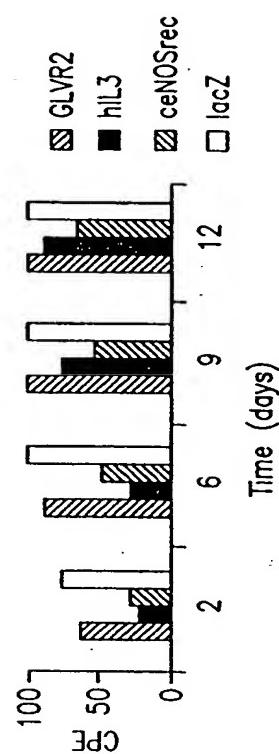


FIG. 37D



Medium changed 7 days after transfection

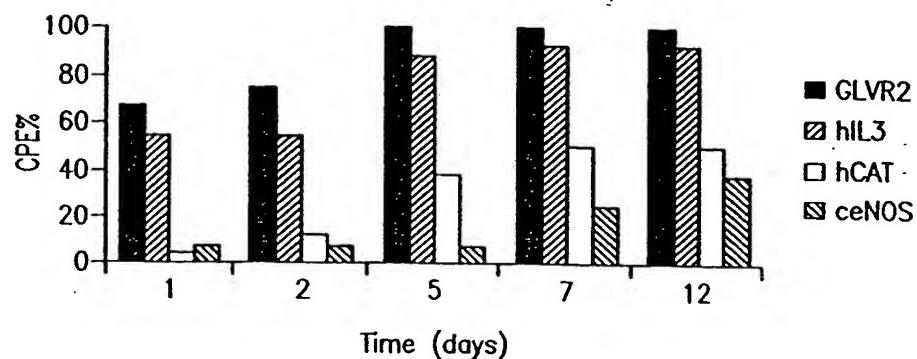


FIG. 38A

Medium not changed

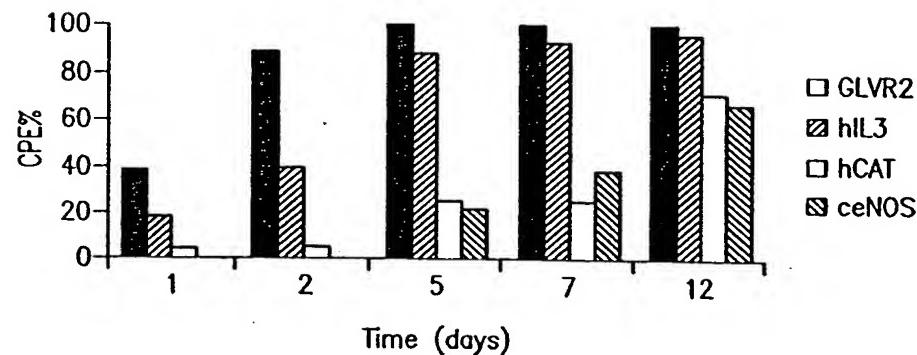


FIG. 38B

Propagation 7 days after transfection

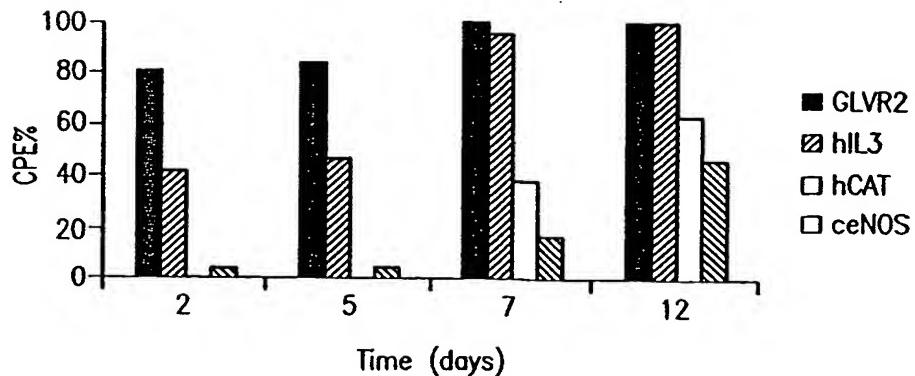
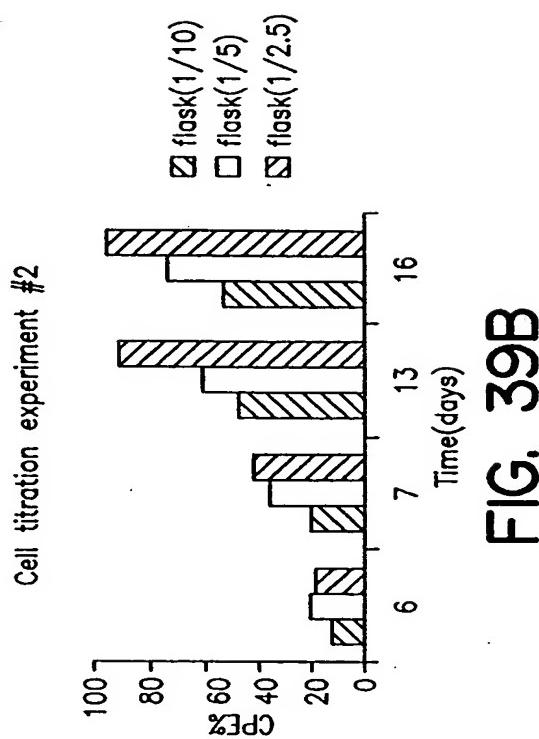
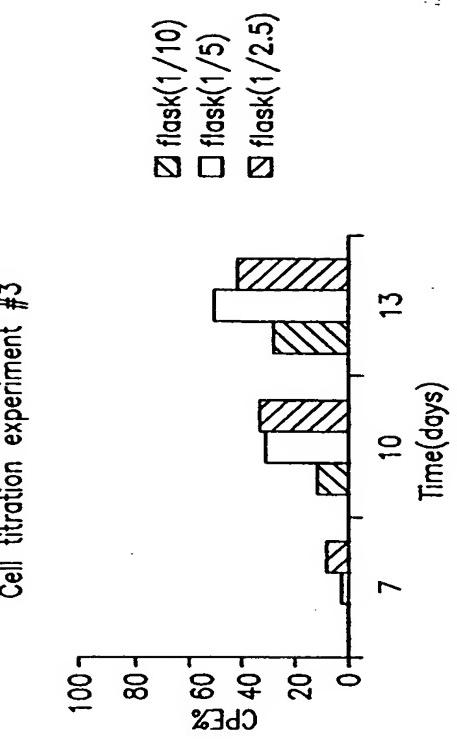
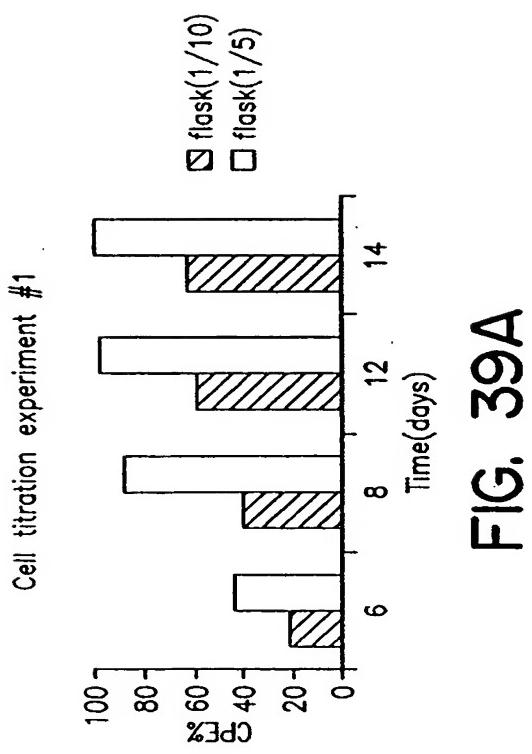


FIG. 38C



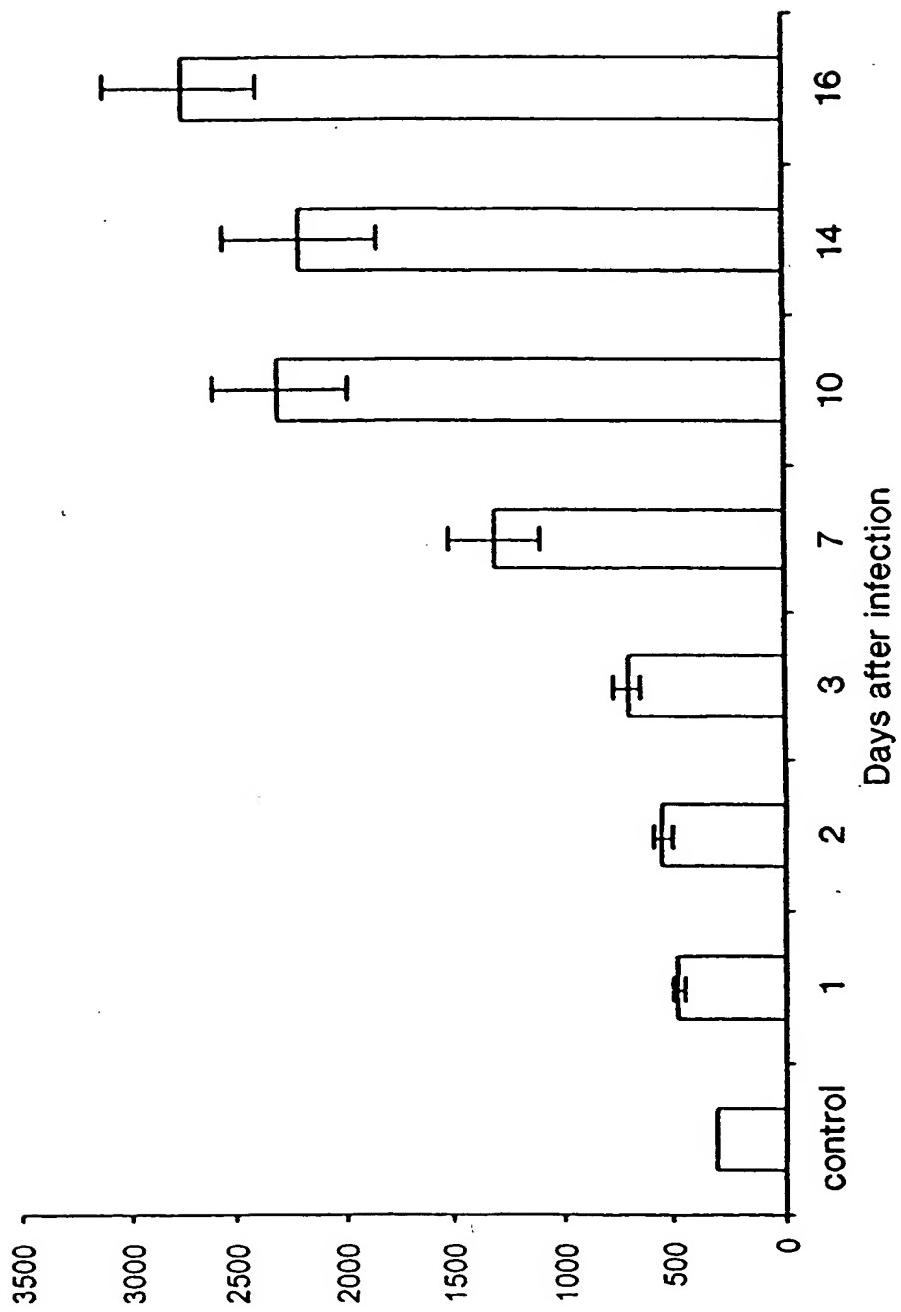


FIG. 40

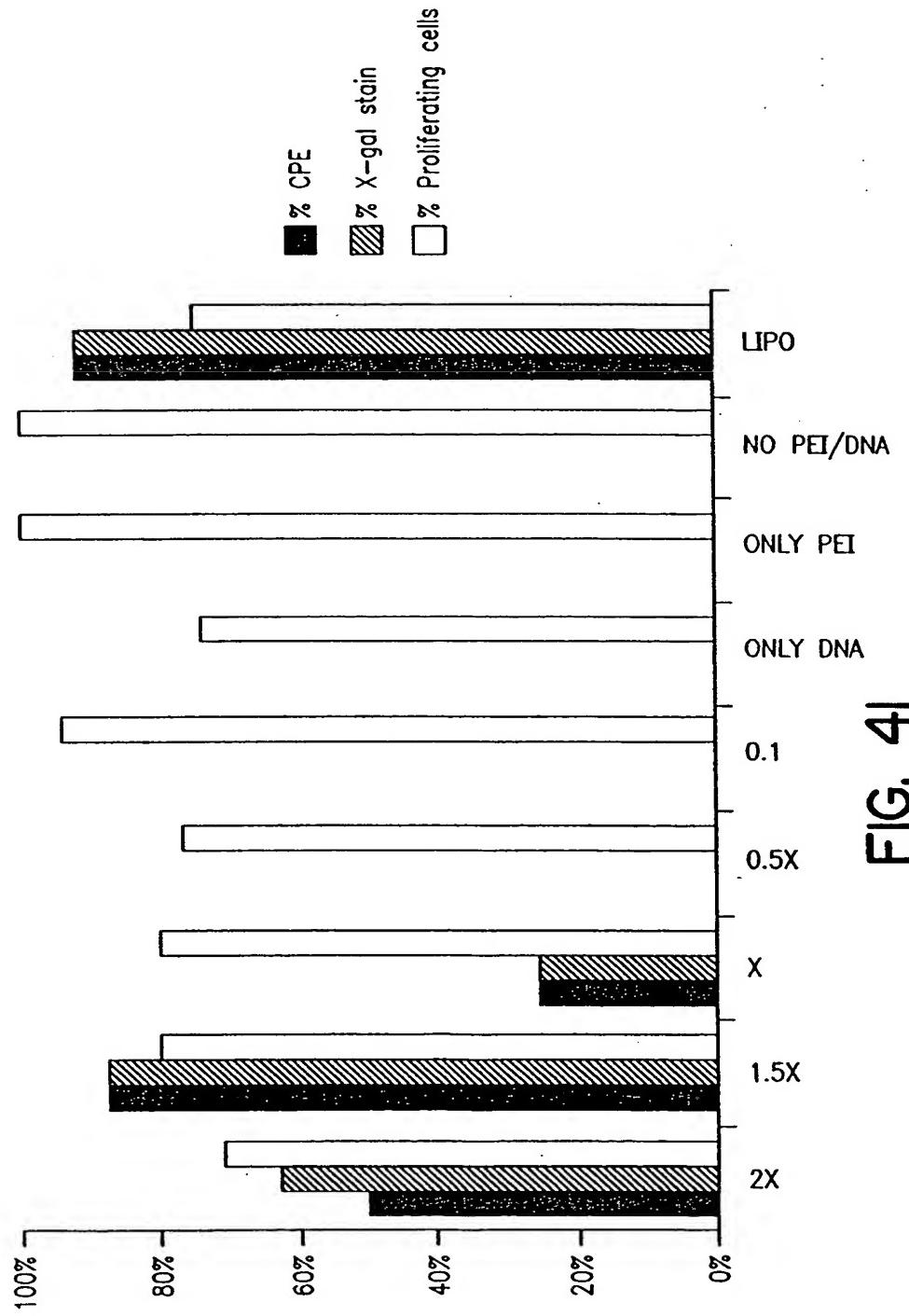


FIG. 4I

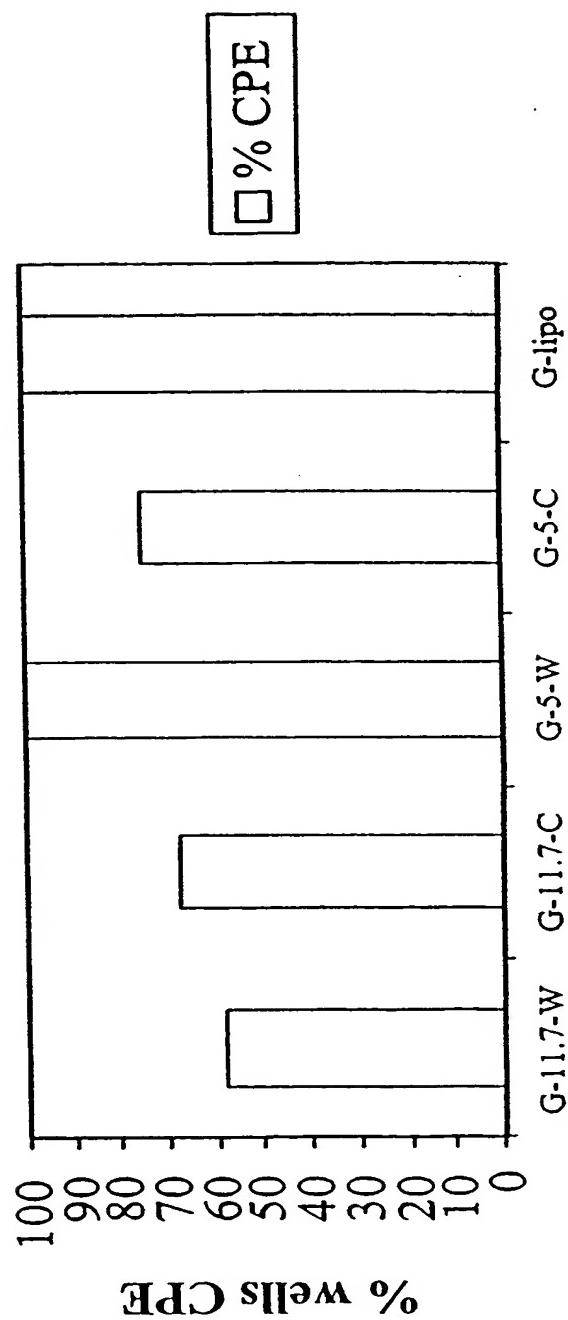


FIG. 42

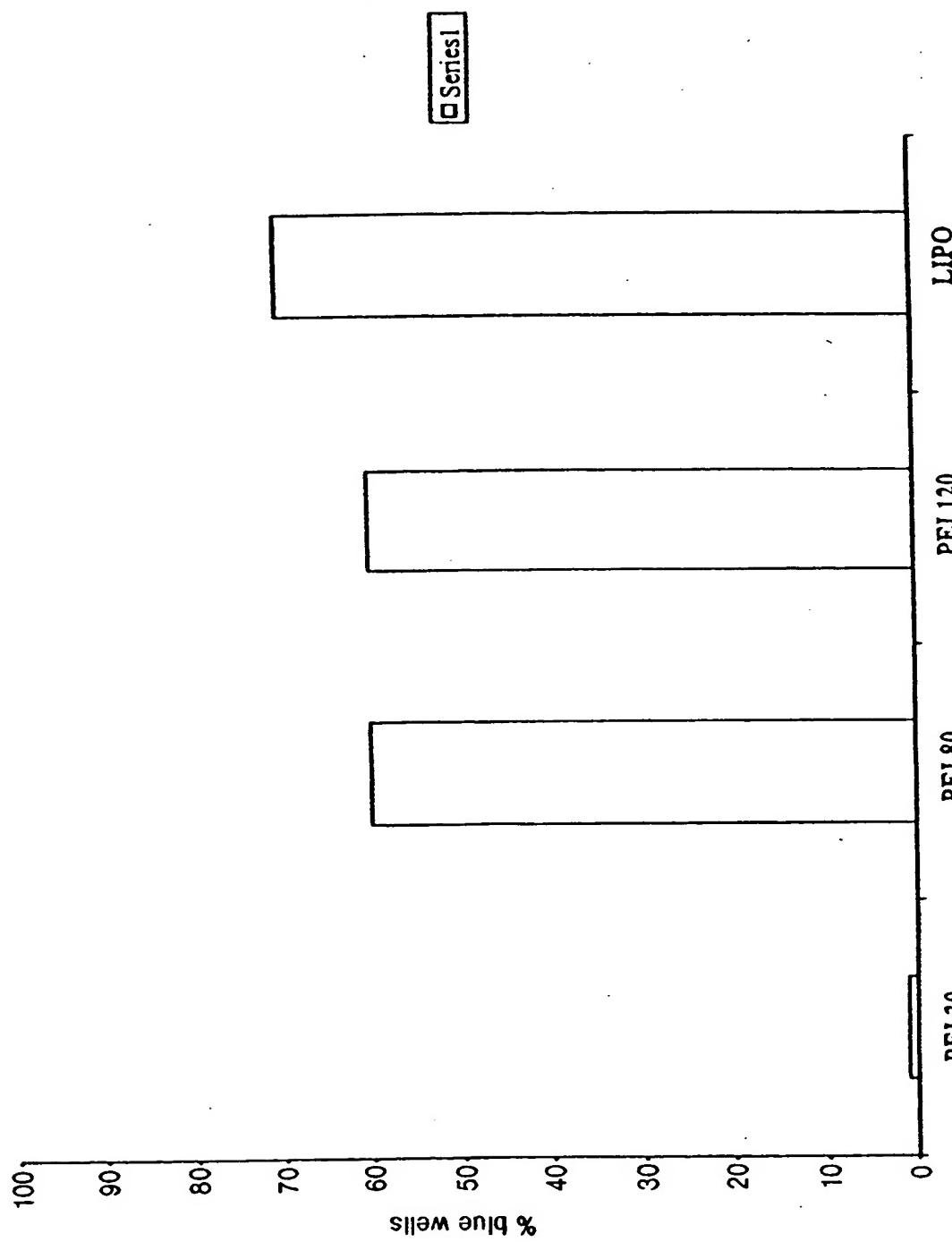


FIG. 43

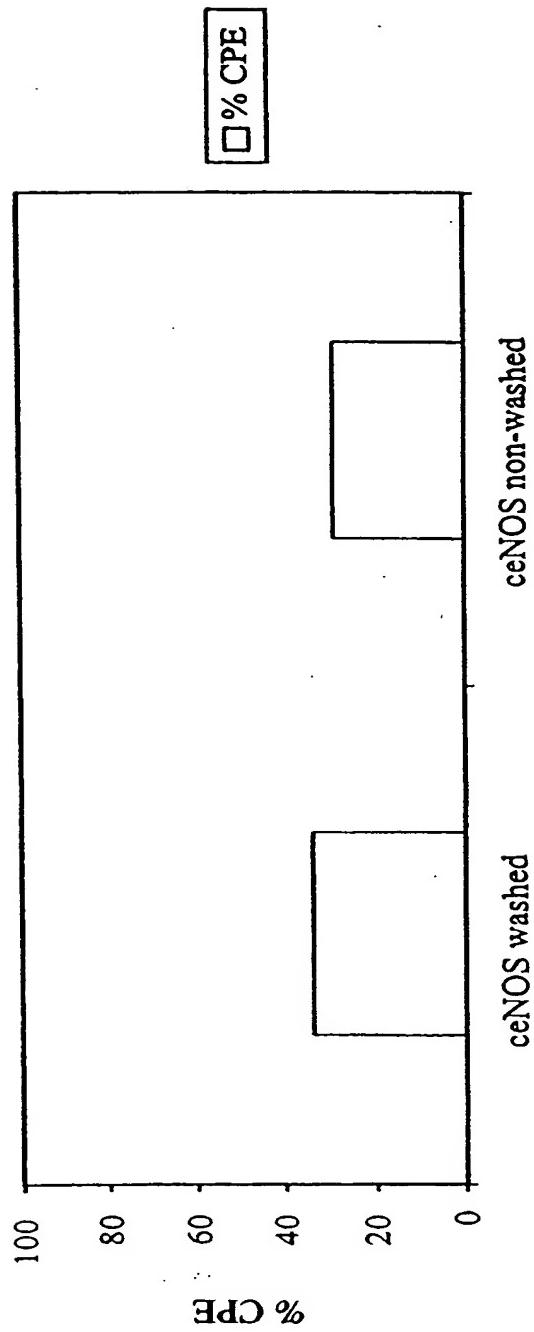


FIG. 44